October 2025

TECHNICAL AND REGULATORY GUIDANCE

BIOLOGICAL CONTAMINANTS OF EMERGING CONCERN (BioCEC)

Prepared By
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Biological Contaminants of Emerging Concern (BioCEC) Team

WELCOME

Disease outbreaks in recent years have expanded public appreciation for linkage between an emerging environmental hazard and health outcomes. Oftentimes, there is not sufficient information to gauge health risk from emerging biological contaminants in the environment nor sufficient collaboration and communication between public health and environmental sectors to coordinate a response. Building a bridge between the expertise of environmental and public health sectors would help to fill this gap.

This guidance document about biological contaminants of emerging concern (BioCEC) is intended for public health and environmental professionals and is rooted in the "One Health" framework. It aims to broaden and deepen technical knowledge and expedite quality regulatory decision-making while protecting human health and the environment. It builds upon the Contaminants of Emerging Concern (CEC) Framework that was published in December 2023 and is meant to provide guidance for states to identify and evaluate the broad range of contaminants and pathogens covered by the term BioCEC.

BioCEC are diverse, and the risks they pose are varied; therefore, it is important to note that this guidance is not comprehensive and will not identify specific risks. This guidance can, however, aid entities as they assess the risk for their unique circumstances. The scope of this guidance is driven in part by the ITRC team's capacity and the expertise of the team members/volunteers.

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Biological Contaminants of Emerging Concern (BioCEC)

Technical and Regulatory Guidance

October 2025

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1 INTRODUCTION TO BIOLOGICAL CONTAMINANTS OF EMERGING CONCERN (CEC) GUIDANCE

1.1 Introduction

This document is intended as guidance to help state environmental and health officials address **biological contaminants of emerging concern (BioCEC)**. It builds upon the Contaminants of Emerging Concern (CEC) Framework that was published on the Interstate Technology and Regulatory Council (ITRC) **website** in December 2023 and is meant to provide guidance for states to identify and evaluate the broad range of contaminants and **pathogens** covered by the term BioCEC. BioCEC are diverse, and the risks they pose are varied; therefore, it is important to note that this guidance is not comprehensive and will not identify specific risks. This guidance can, however, aid entities as they assess the risk for their unique circumstances. The scope of this guidance is driven in part by the ITRC team's capacity and the expertise of the team members/volunteers.

ITRC's earlier Contaminants of Emerging Concern (CEC) Framework was meant to inform environmental regulatory agencies and other stakeholders about examples of existing CEC monitoring programs, point to key variables to consider when evaluating potential **toxicity** and exposure, help communicate real and perceived risk from CEC to the public, and understand how laboratory analytical methods can be used in the identification process. For this guidance, the CEC team adopted the following definition of a CEC:

> **ITRC CEC Definition:** CEC are substances and microorganisms including physical, chemical, biological, or radiological materials known or anticipated in the environment, that may pose newly identified risks to human health or the environment.

BioCEC is included in the ITRC CEC definition, but the biological element is primarily addressed in the Analytical Methods Factsheet on a general basis. BioCEC are addressed more directly through this separate effort and guidance. For this guidance, the BioCEC team adopted the following definition for a BioCEC:

> **ITRC BioCEC Definition:** A microbial pathogenic agent that may pose newly identified risks to humans through the environment and is found in a vector, water, soil, waste, or air.

The traditional definition of the environment includes soil, water, and air, also referred to as **environmental media**, which can be further subcategorized based upon characteristics such as use and location. The definition of an environment in a BioCEC context can be more expansive, depending upon the scenario. **Vectors**, waste, the built environment, and biota can be treated as **sources**, environmental media, mechanisms of environmental transmission, or any combination of these categories. See **Defining the Environment in the Conceptual Exposure Model** section for more information about defining the environment.

The primary audience for this BioCEC guidance is environmental and health professionals at state agencies who are tasked with making informed and timely decisions regarding BioCEC. The content is intended to be useful to environmental consultants and parties responsible for implementing BioCEC programs and interfacing with regulatory agencies regarding BioCEC. It can be useful for federal agencies, industrial representatives, tribal organizations, and other stakeholders who need to manage BioCEC. The subject matter audience for this guidance may differ from the audience for ITRC's first CEC guidance, which focused on chemical CEC and was largely within the scope of environmental professionals.

1.2 Background

There is no denying that microorganisms have provided modern society with significant benefits in industrial, biotechnological, and agricultural settings. One of the major challenges associated with microbiology and public health is managing and reducing the risk associated with the transmission of harmful microorganisms, known as pathogens, and their resulting **infectious diseases** (Haas et al. 2014). Microorganisms generally include all small biological entities that cannot be seen with the naked eye (e.g., viruses, bacteria, fungi, **archaea**, protozoa, algae). They can range in size from 20 nm to 300 µm. They exhibit complex organizational structures that allow them to reproduce using DNA and RNA. Viruses need a **host** cell to reproduce (Mara and Horan 2003).

An infectious disease is an illness caused by the transmission of a pathogen from an infected host to a susceptible host either directly (e.g., person to person) or indirectly (by insects or other animals, or through air, water, food, waste, or soil). The term **communicable disease** usually refers to transmission from person to person or from animal to person (e.g., body fluids, droplets). While all communicable diseases are infectious, not all infectious diseases are communicable (e.g., tetanus is an infectious but not communicable disease). In resource-limited settings, communicable diseases remain a significant cause of mortality and morbidity in all age groups; in high-resource settings, infections associated with the respiratory tract are the most common diseases to impact susceptible populations.

Although pathogenic agents can cause harm to non-human receptors, the scope for this guidance is limited to addressing harm to humans. This guidance focuses mainly on microbial pathogens in environmental media (e.g., surface water, groundwater, wastewater, soil, waste, and air) that can harm humans. For the most part, this guidance does not focus on transmission from human to human (i.e., anthroponotic), animal to human (i.e., zoonotic), or human to animal (i.e., reverse zoonotic), except for transmission from animal waste and vector transmission between animals and humans.

BioCEC may pose newly identified risks to human health or the environment; hence, they could represent an emerging concern. There may also be cases where BioCEC could instead be reemerging. Climate change and anthropogenic impacts on environmental systems create niches for pathogens to survive. Climate change and thawing/melting environments may result in the re-release of organisms that have not evolved with current ecosystems and environmental conditions. Microbes liberated from melting permafrost could be an example of a BioCEC that poses an emerging concern. Warming temperatures may also lead to the migration of invasive species into areas that were previously not as hospitable. It is not well understood how increasing air temperatures, surface water temperatures, precipitation, and flooding might create new environments or more favorable conditions for pathogens to survive. An example of such potential effects of a changing climate is described in **Case Study: Effects of Hurricane Helene on Western North Carolina – Climatic Events and Implications for Biological Contaminants in Drinking Water of this Introduction**, which discusses the impact of Hurricane Helene on the mountains of North Carolina in September of 2024. Additionally, there may be cases where neither the contaminants nor the pathogens are emerging, but awareness of the hazards posed or the fate and transport mechanisms of BioCEC are increasing. For example, antibiotic-resistant bacteria and genes are on the rise in aquatic ecosystems (Water Environment Federation 2023; Garner et al. 2021; Berglund et al. 2023). Anthropogenic activities like agriculture lead to increased nutrient loading, which along with pharmaceuticals in wastewater, may be associated with the introduction of pathogens into the environment. Increased nutrients may also lead to harmful algal blooms. Note that previous ITRC guidance has addressed subjects such as Harmful Cyanobacterial Blooms and their associated toxins (**https://hcb-1.itrcweb.org/**).

This guidance is rooted in the "One Health" approach (**Figure 1-1**), which works to address concerns around infectious disease risk. One Health is a collaborative, integrated, and unifying approach used by the **Centers for Disease Control and Prevention** (CDC), **U.S. Environmental Protection Agency** (USEPA), and **World Health Organization** (WHO) that seeks to optimize the health of people, animals, and

ecosystems while recognizing our interconnectedness and interdependence. One Health involves a holistic approach to addressing potential BioCEC concerns including food and water safety and may be helpful in detecting and responding to future global health threats. This is especially important as the human population continues to grow and expand into new areas and people live in close contact with wild and domestic animals. The intersection of disciplines from numerous sectors can play an important role in reducing threats from food production and distribution, urbanization, climate change, loss of biodiversity, and the rise of **zoonotic diseases**.

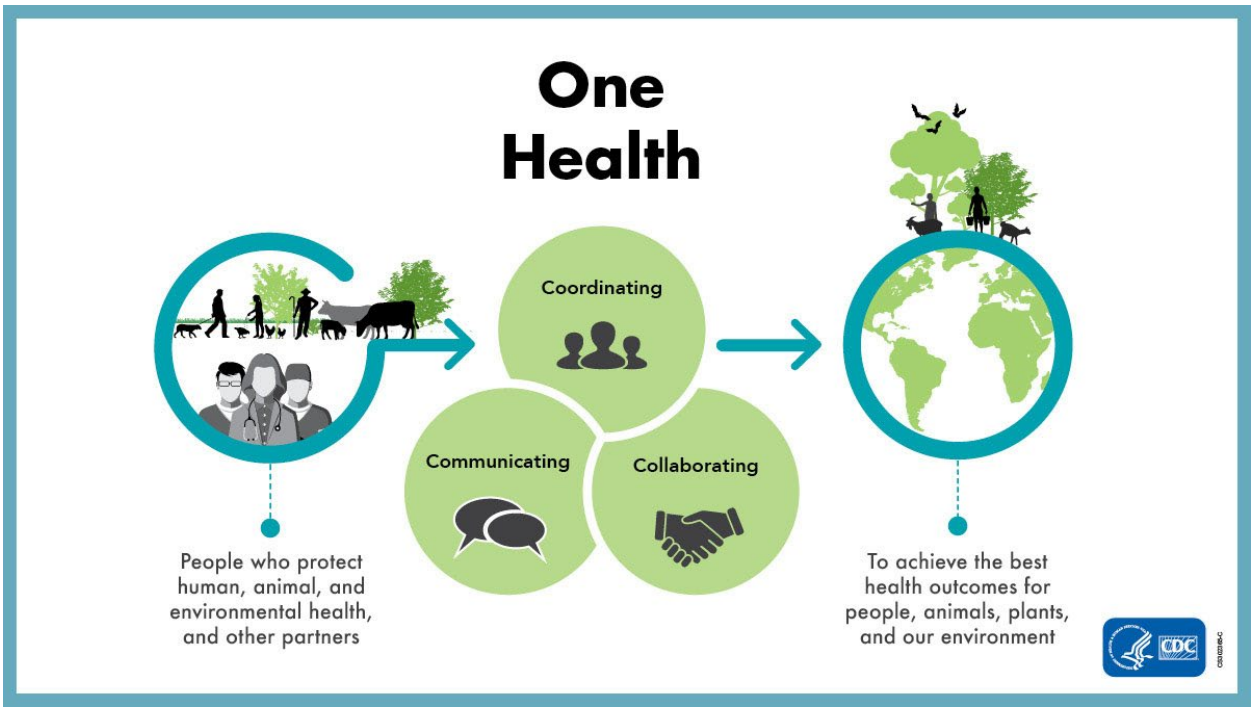

Figure 1-1. One Health approach.

Source: CDC (2024).

1.3 BioCEC Guidance Sections

This BioCEC guidance is divided into several sections to illustrate the totality of this process and how to evaluate each individual segment within the process. The summarized guidance features the following sections: a **Process Guide**, **Conceptual Exposure Models (CEMs)**, **Key Variables**, **Analytical Methods**, and **Monitoring Programs / Resource Hub**. The **Case Studies** section presents several case studies that examine climatic events and the implications for biological contaminants, in addition to the Hurricane Helene case study featured in **Case Study: Effects of Hurricane Helene on Western North Carolina – Climatic Events and Implications for Biological Contaminants in Drinking Water of this Introduction**.

1.3.1 Process Guide

In the **Process Guide** section, we present a guide for identifying BioCEC and actions to take once the presence of BioCEC has been determined. Actions to take include (1) notification and coordination with other agencies, (2) public communication, (3) response, and (4) monitoring/surveillance. Each section is supported by a narrative in a question/answer format that is aligned with available guidance on the topic. Finally, an overview of the contents of this process guide is shown in an accompanying **flow chart**.

1.3.2 Conceptual Exposure Model

A **CEM** is a visual representation of a BioCEC scenario that maps known and potential interactions among an environment, a pathogen, and a host to identify controls that reduce the severity of an outbreak. **The CEM user guide** discusses the **epidemiologic triangle**, which is a model used to describe the interactions among a pathogen, a host, and an environment, and how the epidemiologic triangle can be used to create a CEM. An overview of how regulators can identify contaminant sources, components of the epidemiologic triangle, **cross-media transfer** mechanisms, and exposure scenarios is provided. The benefits and applications of CEMs are presented, with an emphasis on the ability of a CEM to combine interdisciplinary knowledge to create a shared understanding of a BioCEC scenario. This shared understanding guides proactive and reactive regulatory responses. Specific hypothetical CEM scenarios regarding biowaste release to soil, biowaste release to potable water, vector-host interactions, and surface water contamination are presented as examples to inspire regulators for their own CEM creation. Additionally, this guidance includes **a case study that examines an *E. coli* outbreak linked to romaine lettuce** to help illustrate a real-world example of how a CEM can be applied.

1.3.3 Key Variables

Identifying key variables that predict increased potential of environmental transmission of pathogens is crucial to minimize or prevent the harmful human effects of BioCEC. The objective of this section is to characterize key variables that may be used to identify, evaluate, and prioritize BioCEC. In addition, resources that can be used for prioritization of BioCEC are summarized to help assess and mitigate risks of BioCEC and inform decision-making. The **Key Variables** section summarizes the epidemiological triangle (sometimes known as the disease triangle), which is the framework used for the discussion in the section. Key variables that fall under pathogen, environment, and host are identified and described to provide context for the evaluation of risk of BioCEC. Different prioritization schemes that are used to evaluate risk of BioCEC is reviewed, including Health Canada guidance on quantitative microbial risk assessment (QMRA), WHO's guidance, and USEPA's Contaminant Candidate List 5. A process description and **flow chart** developed by ITRC's BioCEC team is included to summarize tools used for prioritization of BioCEC. The **Case Studies** section presents two case studies on the use of QMRA to evaluate the risk of BioCEC: **one case study on the risk of salmonellosis from alternatively produced broiler meat** and **one case study that uses QMRA for direct potable water reuse treatment targets in California**. Additionally, **Risk of *Legionella* Infections from Two Shower Exposure Models in the Case Studies** section includes a case study that examines the risk of *Legionella* infections from two shower exposure models to help illustrate the process of identifying key variables to better understand the environmental transmission of pathogens.

1.3.4 Analytical Methods

Reliable analysis of a pathogen in various environmental matrices and vectors requires a high degree of confidence in its identity and quantification. Microbiological detection and quantification methods are typically developed in and tested across various laboratories and groups with well-defined method limitations and appropriate quality control (QC) practices (see **ITRC Environmental Molecular Diagnostics Section 10 for QC considerations**). These standardized microbial methods usually represent the best technique currently available for the detection and quantification of a specific pathogen (i.e., targeted analysis). To some degree, these standardized methods can be used or modified in some way to capture new groups or subtypes of known pathogens (i.e., suspect screening). For a BioCEC, reliable and standardized analytical methods may not be readily available, especially for new pathogens that have not been previously encountered (i.e., non-target analysis). Applications of the various methods described for targeted, suspect screening, and non-targeted analysis are discussed. The **Analytical Methods** section describes various methods that could be used for detecting and quantifying BioCEC in various environmental matrices and vectors. Not discussed in that section is the increasing use of data analytics

(for example, machine-learning approaches) for monitoring and forecasting contamination in the environment. Reliable detection and quantification of BioCEC is needed prior to applying data analytics.

1.3.5 Monitoring Programs / Resource Hub

The BioCEC team created an **Excel spreadsheet** and narrative highlighting existing monitoring programs, primarily those developed by state governments but also including several federal and international programs. The narrative includes descriptions of the format, findings, significance, and key monitoring programs and resources. The spreadsheet consists of two pages. The first page provides 59 examples of monitoring programs for BioCEC throughout the United States as well as several key international programs. While the list of monitoring programs is illustrative and is not meant to be comprehensive, a minimum of two monitoring programs was chosen to represent states in each of the US regions, as defined by the USEPA. The second page consists of a resource hub with links to websites, primarily from governmental organizations. Please note that, consistent with the overall BioCEC guidance document, the spreadsheet does not include monitoring programs or resources pertaining to indoor air quality or other controls related to the built environment.

The programs reviewed in the spreadsheet pertain to an extensive range of pathogens. These programs cover a variety of sources where pathogens have been detected, including environmental media (such as soil, water, and air), vector populations (such as mosquitoes), human and animal tissues, and healthcare data systems. The spreadsheet reflects the significant role of these monitoring programs in supporting early detection, surveillance, and response to emerging public health threats. The spreadsheet provides examples for professionals who are interested in formulating their own monitoring programs, as well as an overview for general edification regarding what sorts of monitoring programs already exist within the United States, and to a limited capacity worldwide.

1.3.6 Case Studies

Several case studies are featured to provide context to the discussions presented in the other sections of this guidance. The case studies are meant to help illustrate that although BioCEC are complex and situation specific, the CEM, process, variables, factors, and methods discussed in this guidance can assist in the systematic evaluation and prioritization of BioCEC. The case study of Hurricane Helene provided below is illustrative of concepts related to climate change.

Links to the other case studies in the BioCEC guidance:

- **Blastomycosis outbreak**
- ***E. coli* outbreak linked to romaine lettuce**
- **Risk of salmonellosis from alternatively produced broiler meat**
- **Risk of *Legionella* infections from two shower exposure models**
- **Using QMRA for direct potable water reuse treatment targets in California**

1.4 Case Study: Effects of Hurricane Helene on Western North Carolina — Climatic Events and Implications for Biological Contaminants in Drinking Water

1.4.1 Background

Extreme weather events often have adverse impacts on drinking water supplies and sanitation resources, which increases the risk that communities will be exposed to biological contaminants. This case study describes the effects of Hurricane Helene on Western North Carolina and particularly the drinking water system in Asheville, North Carolina, which was severely damaged by the storm.

Federal, state, and local agencies and organizations have faced challenges in recent years related to the increased intensity and frequency of events such as forest fires, hurricanes, and cold weather storms. Such events increase the risk of the occurrence of and exposure to a variety of biological contaminants. Tools such as the **CEMs**, **analytical methods**, and analysis of **key variables** provided in this BioCEC guidance provide important support to the necessary response actions.

On September 27, 2024, heavy rain and high winds associated with the remnants of Hurricane Helene, which made landfall on the Gulf Coast the day before, moved through Western North Carolina. Record-breaking rainfall totals ranged from 12 to 20 inches across Western North Carolina. The storm caused widespread flooding, damaged infrastructure, and left large swathes of the area without power, water service, or communications by cell phone and internet (**Figure 1-2**). The storm event and associated flooding caused an estimated $59 billion in damages and washed away many bridges, homes, businesses, and roadways (NC OSBM 2024). This case study focuses on the drinking water supply for the City of Asheville, North Carolina, but it should be noted that other public systems in Western North Carolina, including Banner Elk and Spruce Pine as well as many private well owners, also suffered catastrophic damage and were forced to discontinue water service for extended periods.

Figure 1-2. Aerial photo of floodwater inundating the Asheville River Arts District along Foundy Street on September 27, 2024.

Source: Time Reaves Photography 2024 used with permission.

In the days before the Helene storm system moved through Western North Carolina, a separate storm system had dropped substantial rain across the area. Combined, the two weather systems led to 104 people losing their lives because of floodwaters, landslides, or other fallout conditions from the storm (**Hurricane Helene Storm Related Fatalities | NCDHHS**). Many public services were overwhelmed by the scope of the disaster. Public water systems were heavily damaged in Asheville, North Carolina, a city of 95,000 people (based on data from census.gov), as well as other communities across the region (J. Silver, Buncombe County Health Department, interviewed by J. Mahan, March 17, 2025). Mission Hospital in

Asheville was forced to use emergency generator power and lost water service as well as communications by internet, rendering electronic medical records systems inoperable. Road systems were damaged to such a degree that in the immediate aftermath of the storm the North Carolina Department of Transportation issued a statement indicating that all roads in Western North Carolina should be considered closed (NCDOT 2024).

1.4.2 Damage to Water Treatment Infrastructure

The effects of Helene required a coordinated response by aid agencies to assist Western North Carolina with recovery. Relevant agencies included federal (USEPA, the Federal Emergency Management Agency, the US Army Corps of Engineers), state (North Carolina Department of Environmental Quality, North Carolina Department of Health and Human Services), and local (Buncombe County Health Department, Asheville Water Resources Department [WRD]). The municipal water system in Asheville is managed by the City of Asheville's WRD. The WRD system consists of three drinking water plants (North Fork, William DeBruhl, and Mills River) and includes more than 1,700 miles of water distribution lines. The North Fork plant located 15 miles from downtown Asheville is the largest of the three treatment plants and supplies most of the water to the Asheville area. The most immediate challenge to the Asheville WRD from Helene was that the North Fork water treatment plant and associated distribution system were heavily damaged. Another significant challenge faced by Asheville WRD was that sections of the 36-inch water main that provides water from the North Fork plant to Asheville had been washed away by floodwaters.

1.4.3 Water Quality and BioCEC Concerns

Landslides, fallen trees, and stormwater runoff led to turbidity levels in the North Fork Reservoir that the plant was not designed to handle. Historically, water obtained from the North Fork Reservoir has been of excellent clarity with a Nephelometric Turbidity Unit (NTU) of 1.0; however, after the storm, turbidity was as high as 80 NTUs. The damages to infrastructure posed an increased risk of exposure to waterborne and related pathogens to Asheville and Western North Carolina residents.

The Helene storm event fits into the pattern of tropical storms detailed by researchers Victoria Lynch and Jeffrey Shaman in an article from *PLOS Water*. The authors report that these storms lead to an increase in the occurrence of hospitalizations due to waterborne infectious disease. Specifically, the incidence of Legionnaires' disease, *E. coli*, and cryptosporidiosis incidence increased after intense rainfall events (Lynch and Shaman 2023; 2024). The increased illness is attributed to factors such as sewage system overflows or bypasses and mobilization of pathogens from soil and sediment by overland water flow. Pathogens associated with waterborne illness include the following:

- Biofilm-forming bacteria — Nontuberculous mycobacteria, *Pseudomonas*, and *Legionella*
- Bacterial — *Salmonella*, Campylobacter, *Shigella*, and *E. coli*
- Parasitic — *Cryptosporidium*, *Giardia*, species of amoeba and protozoa
- Viral — Norovirus

1.4.4 Post-storm Mitigation and Recovery

The aftermath of Hurricane Helene presented a situation where there was potential for widespread waterborne illness, but a series of steps taken by the WRD and other organizations helped to avoid such an outcome. Immediate efforts to avoid and mitigate the risk of waterborne illness in Asheville included the following:

- A boil water notice was issued for the Asheville municipal water system and for water obtained from private wells inundated by floodwater.
- Bottled and bulk potable water was made available to residents through the efforts of public and private storm relief groups.
- Public health officials provided guidance to establishments (restaurants, hotels, childcare centers, etc.) on how to operate safely during a boil water advisory or notice.
- Mobile sanitation centers were established to provide opportunities for bathing, brushing teeth, etc.
- A mobile water treatment system was constructed in nearby Swannanoa, North Carolina. A temporary water **intake** was placed in the adjacent Swannanoa River. As there were no regulatory mechanisms to permit this type of system in real time, a system design with a professional engineering certification was accepted and put on file by the local office of the North Carolina Department of Environmental Quality — Public Water Supply Section.
- Testing of public and private water supplies started during the immediate storm recovery period.
- Mission Hospital instituted a system whereby large quantities of potable water were transported from outside the area, offloaded into tanks set up outside the hospital, and pumped into the facility's water system. Many Asheville businesses instituted similar systems so that they could reopen.

After the Helene system moved through on September 27, most of Asheville did not have water service at all (Figure 1-3). The boil water notice was extended and remained in effect until November 18 when repairs and testing of the water system allowed for it to be lifted. A portion of the southern WRD service area remained in service as the Mills River plant did not suffer heavy damage. Drinking water was in short supply until truck deliveries of bottled water could catch up to the needs. Toilets went unflushed, or residents carried water in buckets from nearby streams to flush them. The WRD began feeding water into the distribution system as soon as portions of the system were back online. There was agreement among officials that any water provided through the distribution system was better than none. Therefore, in mid-October the system was pressurized in stages, and residents were able to obtain water from their household taps — much of the water was untreated and/or muddy and not suitable for drinking or cleaning.

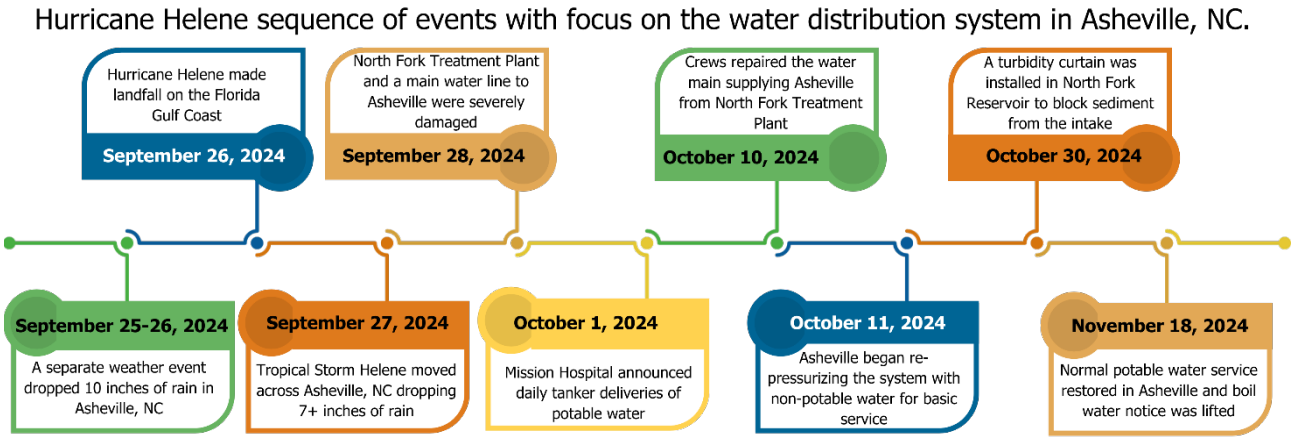

Figure 1-3. Timeline of Hurricane Helene and impacts to the Asheville, North Carolina, water distribution system.

As the WRD re-pressurized the distribution system in mid-October they were not able to use the standard treatment processes. To achieve a basic level of disinfection, chlorine was used at a concentration of 8 milligrams per liter (mg/L) for water as it left the North Fork plant. This resulted in an average concentration of 2 to 4 mg/L across the distribution system (J. Kohn, North Carolina Department of Environmental Quality, interviewed by J. Mahan, December 2, 2024). Residents were able to use the water for bathing, flushing toilets, and washing dishes.

Asheville WRD tested water from the reservoir and throughout the distribution system as service was slowly restored. Concerns for water quality included the potential presence of harmful pathogens, elevated levels of metals, and the potential for elevated levels of disinfection byproducts. WRD conducted testing for lead, copper, aluminum, iron, chlorine, manganese, total coliform, and *E. coli*, as well as turbidity. On November 15, the city announced that water treatment infrastructure, although not fully restored, was repaired to such a degree that sufficient water to service the Asheville system (~25 million gallons daily) was available. Final testing for *E. coli*, coliform, and chlorine residuals was conducted over the next several days. On November 18, normal water service returned 52 days after the storm.

1.4.5 Summary

Actions taken by water authorities and public health officials included dissemination of educational information on the safe use of public water and private well water affected by the storms; construction of temporary sanitation centers, which provided restrooms, drinking water, water for personal hygiene, and water for basic kitchen tasks; and testing of public and private water supplies for waterborne pathogens. While there were individual reports of gastrointestinal illness, the preventive actions taken to improve sanitary conditions were successful in helping to avoid widespread outbreaks of waterborne illness (**Aftermath of Helene making WNC survivors sick | NC Health News**, **Hurricane Helene Update: Death Toll and Unaccounted For | Regional/National Headlines | local3news.com**).

Given the scale of the storm and its aftereffects, this was a victory. The fact that an inland city 400 miles from the coast where Hurricane Helene made landfall was impacted by the storm so dramatically and had to take these kinds of actions to recover from its effects is an indication of how climatic events can challenge the water utility and public health infrastructure.

Information in this guidance document is meant to support efforts to respond to and mitigate the kinds of hazards and risks posed by weather events similar to this one in Western North Carolina. The event in this case study focuses on the potential for waterborne illness; however, the guidance document also addresses potential risks associated with other media including soil, waste material, and air. Additional information and resources related to Hurricane Helene recovery in North Carolina is available at the **North Carolina Department of Health and Human Services website**.

2 PROCESS GUIDE

In this section, we present a process guide for identifying and addressing human health-relevant **biological contaminants of emerging concern (BioCEC)**. For a detailed discussion of the scope of what is considered a BioCEC, please see the **Introduction** section. We begin this section by providing a discussion of the first steps of a BioCEC event: identifying and confirming the presence of BioCEC. Next, we discuss potential actions to take, including notification/coordination with agencies, public communication, and response. Each discussion of potential actions begins with key questions to consider for the category of action. These are followed by a brief narrative discussing an overview of the key questions. An overview of the process described below is outlined in **Figure 2-1**.

2.1 Identification of BioCEC

The identification of BioCEC ultimately stems from monitoring efforts. These range from state and local agencies implementing their own programs to monitor for specific BioCEC of interest in their areas, to monitoring networks that have been implemented by other states or federal agencies such as the Centers for Disease Control (CDC) (e.g., **https://www.cdc.gov/emerging-infections-program/php/about/index.html**). Development of analytical methods to effectively identify BioCEC in certified and commercial laboratories is also an important factor as our understanding of how BioCEC distribute and interact with various environmental compartments and media is refined. Methods for identifying BioCEC are discussed in greater detail in the **Monitoring Programs** and **Analytical Methods** sections of this document. It is important to note that in addition to the identification of a BioCEC, the determination of a high risk of occurrence of a BioCEC may also spur action. For example, in the case

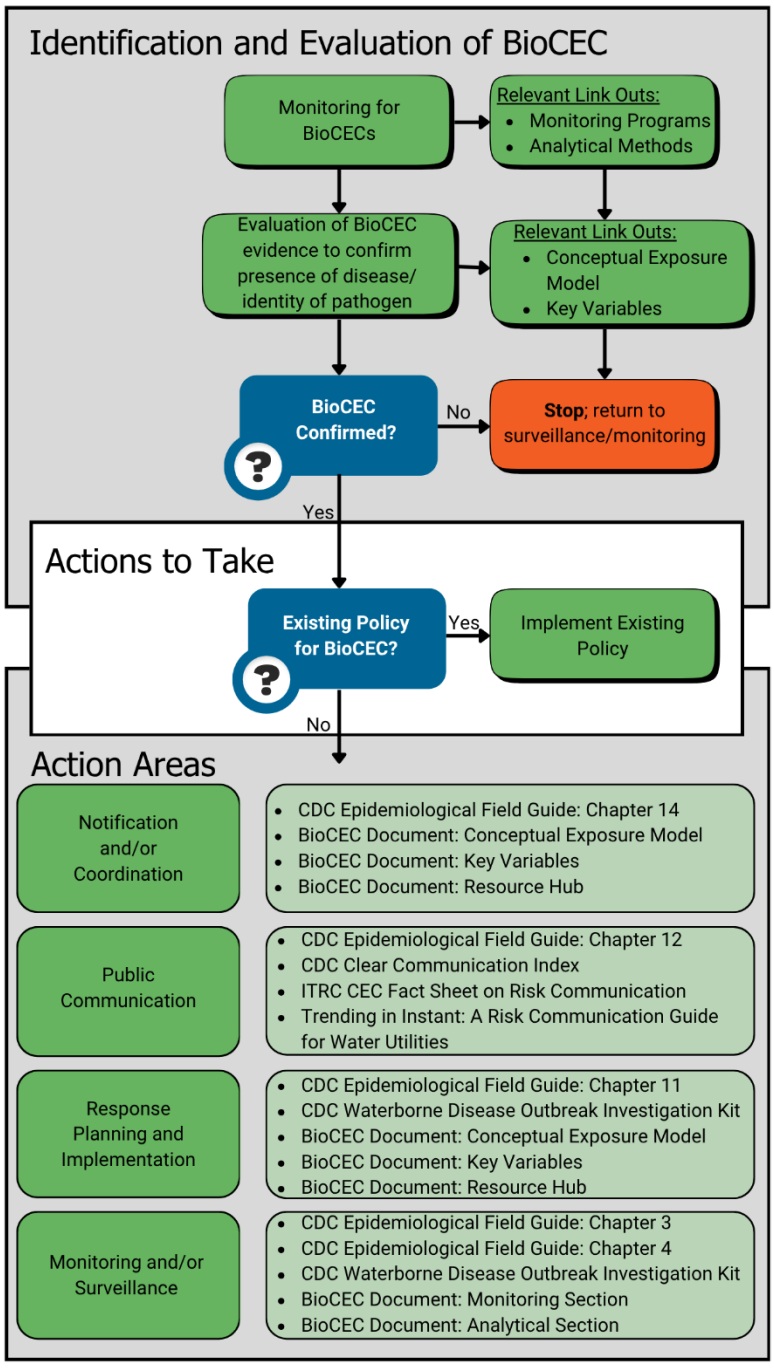

Figure 2-1. Process Guide components, flow, and key references.

study presented in the **Introduction**, the high risk of BioCEC occurring from damage to water infrastructure caused by Hurricane Helene motivated a response instead of the identification of the BioCEC themselves.

2.2 Actions to Take

Once the presence (or the unacceptably high risk) of a BioCEC has been confirmed in a jurisdiction, several potential actions can be taken. A subset of these actions include (1) notification and coordination with state, local and federal entities; (2) communication with the public; (3) BioCEC response; and (4) continued monitoring. Note that the order of actions presented above is not prescriptive, and the actual order of actions taken will depend on the individual situation. In addition, the actions presented here are general categories that are interconnected and likely conducted in coordination with each other. The concepts discussed here are illustrated by a case study of an outbreak of blastomycosis, which is presented in the **Case Studies**.

2.2.1 Notification and Coordination

Other health and environmental organizations and agencies should be notified and coordinated with to plan and carry out a response to a BioCEC. Due to the possibility that a BioCEC outbreak may cross existing program boundaries, new partnerships across organizations and agencies may be warranted.

2.2.1.1 Key Questions

- Is the potential BioCEC outbreak multijurisdictional?
- What local, state, and federal bodies should be contacted to plan a coordinated response?
- How should public communications be coordinated across jurisdictions?
- Have existing partnerships been established between relevant organizations and agencies, or do new lines of communication need to be established between points of contact?
- In what media can the suspected BioCEC be found in the environment?
- Through what media does transmission of the BioCEC occur?
- Does comprehensive guidance for responding to this type of BioCEC already exist?
- If guidance does not currently exist, are there resources available to help create a response protocol?

2.2.1.2 Discussion

Efficient and effective coordination with relevant agencies is critical for successfully navigating BioCEC outbreaks and contamination events (hereafter simply BioCEC events). The jurisdictional extent of the BioCEC event is a key piece of information that will guide coordination moving forward with the response to the BioCEC. This can be influenced by the category of the **exposure medium** (e.g., food vs. water), the medium in the environment in which the BioCEC can be found, and the identified spatial extent of the BioCEC.

The majority of BioCEC events are identified and investigated at the local and state level (see the examples in the **Case Studies**), but subsequent investigation may lead to the involvement of several other

agencies at different levels of regulatory authority. For example, a BioCEC linked to illness in a single restaurant may be under the jurisdiction of the local or state health authority. A BioCEC spread by contaminated water onto fresh produce may span state lines, resulting in a multijurisdictional situation. A real-world example of such a situation is provided in a case study of a BioCEC event involving contamination of romaine lettuce (see **Case Study: Blastomycosis Outbreak**). Identifying the key variables (see the **Key Variables**) and an appropriate **conceptual exposure model (CEM)** (see the **Conceptual Exposure Model**) of the BioCEC can help identify relevant agencies.

Once the jurisdictional extent of a BioCEC event has been identified, it is important to clearly identify roles for investigating, responding to, and communicating about the BioCEC. Investigations should be coordinated at the level where relevant investigation steps can be most effectively implemented. This requires identifying the agency with sufficient resources, expertise, and legal authority to collect, organize, analyze, and disseminate information. When these characteristics are not shared by a single agency, defining clear roles for organizations is critical. A system such as the incident command structure, a framework commonly used by emergency response organizations to manage incidents (FEMA 2024) can be helpful in this coordination. Limited points of contact and clear communication trees should be identified between agencies. BioCEC events may cross over existing program boundaries and require new partnerships to be established with various agencies and organizations. Any new partnerships should be identified and established as soon as possible to reduce any delay in communication and coordination.

The ultimate success of coordination should be measured by the ability to identify the pathogenic agent, mount a prompt public health intervention, and effectively control the outbreak. Much of the above information was summarized from the **CDC Field Epidemiology Manual: Chapter 14** (Rasmussen and Goodman 2019); please see this reference for more details on coordination and notification between agencies. A list of federal and international agencies frequently involved in BioCEC events can be found in the **Monitoring Programs**. For coordination and notification at the state level, please refer to information available on individual state health agency websites.

2.2.2 Public Communication

Effectively communicating with the public is a crucial piece of planning and implementing responses to BioCEC.

2.2.2.1 Key Questions

- What role should my agency play in public communication?
- Is there a public communication timeline? Should communication be delayed or expedited?
- How much technical detail should be included in communication?
- What are the pitfalls of public communication?

2.2.2.2 Discussion

Public communication encompasses a variety of activities, such as press releases, internet social media posts, news conferences, and fact sheets. Although different in form, medium, and content, each ultimately aims to communicate information about the potential for public health risk and should be timely, clear, accurate, and concise.

A primary goal of public communication about BioCEC is to help people understand the risks posed by pathogenic agents and to provide enough detail so they can avoid adverse outcomes. One challenge is

simplifying complex technical information into the key components that are both accurate and interpretable by the intended audience. Another important challenge, however, is that risk is perceived differently by different members of the public. A detailed discussion of risk perception is beyond the scope of this guidance (see Rasmussen and Goodman 2019, Table 12; Lohiniva et al. 2022), but in general, a person will change their behavior in response to a perceived risk if they feel that the risk posed is unacceptable. While this judgment involves several interacting factors (Rasmussen and Goodman 2019, Table 12.1), people are more willing to accept a risk (that is, not change their behavior in the face of a risk) the more familiar they feel with the situation. The converse is also true: people are generally less willing to accept risks if they feel less familiar with a situation. Another key challenge is to convince people to accept the information they are given. People are generally more likely to accept an information source if that source is perceived to be competent, empathetic, and honest. Finally, a goal of communication is to convey information that resonates with people in a way that helps them understand it and is relevant to their situation.

A more detailed discussion of risk communication can be found in the Interstate Technology and Regulatory Council (ITRC) **Contaminants of Emerging Concern (CEC) Fact Sheet on Risk Communication** (ITRC 2023).

It is important that the roles and responsibilities for public communication about BioCEC are determined as soon as possible in the BioCEC response process and that those roles are clear and consistent. If possible, public communication roles should be determined prior to the emergence of a BioCEC so that agency roles are clear from the beginning. Although there is no timeline for public communication of BioCEC, effort should be made at the beginning of the response process (and refreshed throughout) to gain situational awareness to improve communication throughout the response. Such activities could include the following:

- Identifying target audiences and key demographics that may be affected by a BioCEC.
- Identifying how information is consumed within different communities (geographical, cultural, religious).
- Identifying key behaviors influencing risk and developing messaging to address these behaviors.
- Identifying potential barriers to communicating technical information (i.e., language) that need to be overcome.
- Identifying potential barriers (i.e., cultural) to responding to BioCEC.
- Building relationships and communication with key partners, such as organizations, leaders, and influencers.
- Gauging audience perceptions of how communication is received.

It is critical that public communication practices build trust between the public and health authorities over the timeline of the BioCEC response process. This is complicated by uncertainty on the part of the authorities about the current status of the BioCEC event and its trajectory. As part of maintaining and building trust with the public, it is important that this uncertainty is communicated clearly.

Finally, when communicating with the public, common pitfalls to be avoided include the following:

- Not being honest OR not being perceived as honest through poor communication practices
- Not communicating empathy

- Inconsistent messaging
- Unclear messaging

More detailed information on communication during a BioCEC event can be found in the **CDC Field Epidemiology Manual: Chapter 12** (Rasmussen and Goodman 2019). A detailed guide to developing written public communication products for communicating public health information is provided by the **CDC Communication Index** (2019). In addition to the **ITRC CEC Risk Communication Fact Sheet** (ITRC 2023) noted above, detailed guidance for risk communication for public water system providers is provided by the American Water Works Association (AWWA 2019).

2.2.3 Response Planning and Implementation

A response is the technical plan to address the BioCEC and its effects. Implementing an appropriate response is essential to mitigate risk to public health.

2.2.3.1 Key Questions

- What kind of response is warranted for the BioCEC? Is the response based on established knowledge?
- Are there existing CEMs that could apply or serve as a basis for developing a CEM for the BioCEC? (See the **Conceptual Exposure Model**)?
- What is the severity and frequency of the risk to public health due to the BioCEC?
- What basic biological and/or epidemiological information is needed to formulate a proper response? If information is missing, are proxies available?
- Will the exposed population build immunity, and if so, over what time frame?
- Are vaccines or prophylactic therapies available?
- Are there agencies in addition to those identified above that are needed to physically respond to the BioCEC?
- What elements of the response will differ between the short-term (e.g., emergency) and long-term phases of a BioCEC event?
- What will be the roles of the various coordinating agencies in the response?
- Will there be a role for the public in the response?
- How will the response change with evolving information?
- How will the BioCEC response be funded?
- Are there regulatory criteria that trigger environmental responses?

2.2.3.2 Discussion

Implementing a comprehensive response plan is essential for mitigating the public health risk associated with exposure to BioCEC. Public health responses should be scientifically driven by established facts and data, knowledge from previous investigations, and current investigation findings. For detailed information on establishing the scientific basis of a BioCEC event, see discussions in the **Conceptual Exposure Model**, **Key Variables**, and **Analytical Methods** sections. Adapting components of the response may be necessary as information on the BioCEC and the outbreak evolves. Responses to emerging contaminants benefit greatly from open, two-way communication between governmental agencies and the public. Especially in cases when knowledge on the BioCEC is limited and information on how to respond to an outbreak is continually changing, communication with the public is essential to retain public trust in responsible agencies.

There are many factors to consider when planning a response, including the type of BioCEC driving the event, the **sources** of the BioCEC, and modes through which the BioCEC can spread. Additionally, the potential severity of the problems caused by the BioCEC will inform agencies on how quickly practices should be implemented. Please refer to the **Key Variables** and **Conceptual Exposure Model** sections for a more detailed discussion of **pathogen** characteristics and pathogen–**host**–environment interactions. These characteristics are important to understand in order to effectively plan and execute a response. An effective response to a BioCEC would likely involve an approach with multiple components including a robust monitoring program, development of mitigation strategies, open communication with stakeholders, and the implementation of regulatory actions designed to reduce exposure and transmission. It is important to determine whether an existing response plan is available with local governmental agencies or whether a plan needs to be developed. Similarly, if a BioCEC is relatively common in another part of the world but is newly emerging in the United States, there may be some existing guidance for developing a high-level response. Alternatively, if the BioCEC is closely related to a BioCEC that has been seen in the past (e.g., a newly emergent variant from a family of viruses previously linked to an outbreak), it is possible that existing guidance and resources used for that previous BioCEC can be used as a starting point for developing a response to the pathogen.

Please refer to the **Monitoring Programs / Resource Hub** for links to federal programs and lists of outbreaks, as well as a World Health Organization list of **vector-borne diseases**. For a detailed discussion of this topic, please see the **CDC Waterborne Disease Outbreak Investigation Toolkit** (CDC 2024), and the **CDC Field Epidemiology Manual: Chapter 11** (Rasmussen and Goodman 2019).

2.2.4 Continued Monitoring

During and after a BioCEC response, monitoring can be carried out to provide information about the extent (geographic, population impacted) and severity (health impacts, including death) of the identified BioCEC, as well as to evaluate the extent to which the public health response in addressing the BioCEC has been successful. Please refer to the **Monitoring Programs spreadsheet** for examples of existing monitoring programs.

2.2.4.1 Key Questions

- Can the BioCEC be monitored directly or indirectly?
- How can intervention(s) be assessed, both in the short and long term?
- What monitoring resources are available? See the **Monitoring Programs**.
- Are there state, federal, or commercial labs with expertise in monitoring the BioCEC?

- What type of analytical methods are available for monitoring? See the **Analytical Methods**.
- Is there any potential for citizen involvement (e.g., a system to send observations, phone applications for data collection)?

2.2.4.2 Discussion

When considering a continued monitoring plan, an agency may consider whether it is most appropriate to conduct the monitoring on their own, or to partner with a local, state, and/or federal partner. In addition, different monitoring programs may be needed at different stages of a BioCEC event. For example, the type and extent of monitoring used for general BioCEC may be different from the type of monitoring needed once a potential BioCEC is identified.

More information on this topic can be found in the **Monitoring Programs** of this document. Detailed discussions of field data collection and monitoring programs can be found in the **CDC Field Epidemiology Manual (Chapters 4–5)** (Rasmussen and Goodman 2019) and in the **CDC Waterborne Disease Outbreak Investigation Toolkit** (CDC 2024).

3 CONCEPTUAL EXPOSURE MODEL

3.1 Introduction

3.1.1 What Is a Conceptual Exposure Model?

A **conceptual exposure model (CEM)** is a visual representation that maps known and potential interactions among an environment, **pathogen**, and **host**, typically at a local level. A CEM can be presented in various ways, such as through illustrations or block diagrams. A CEM is a critical part of understanding and responding to pathological agents. Hypothetical CEMs can be found in **Conceptual Exposure Model Examples**, and a case study using an extensive CEM can be found in **Case Study: Using a Conceptual Exposure Model to Address a 2018 *E. coli* Outbreak Linked to Romaine Lettuce**.

3.1.2 Defining Pathogen and Host

A pathogen is traditionally defined as an organism that can cause disease in a host, with the severity of the disease symptoms being referred to as **virulence** (Mara and Horan 2003). A host is an organism that harbors a pathogen or parasite. In this guidance, the host refers to a human host that is exposed to or harbors the pathogen. The outcome of the pathogen/microbe-host relationship depends on the **pathogenicity** of the organism, which is the ability of a pathogen to cause disease in a host, and the host characteristics. More information regarding host factors that affect health outcomes and pathogen categorization, biology, transmission, and virulence can be found in the **Key Variables** section.

3.1.3 Defining the Environment

The traditional environment in a CEM consists of soil, water, and air, also referred to as **environmental media**, which can be further subcategorized based upon characteristics such as use and location. The specificity of environmental media categorization depends upon the **biological contaminants of emerging concern (BioCEC)** scenario and the intended audience. Water is often a key component of a CEM, as many pathogens rely on water to reach a host. Given the variability in potential **sources**, a CEM should specify the category of water such as surface water, groundwater, stormwater, potable water, wastewater, gray water, and recreational water. Soil can be described by depth, use, and type. Air can be described by its location, such as indoor air, ambient air, and soil gas.

The environment may be more expansive than environmental media in a CEM and is influenced by the identified scope, scale, and BioCEC characteristics. **Vectors**, the built environment, and biota can be treated as sources, environmental media, mechanisms of environmental transmission, or any combination of these categories, depending on the scenario. Vectors are organisms that transmit a pathogen to a human host and may be visualized as environmental media, a mechanism of exposure, or both within a CEM. For example, a vector that uptakes a BioCEC from breeding grounds and transmits it to humans can be considered an exposure mechanism, whereas a vector that spreads a BioCEC to new breeding grounds acts as a contaminated environmental medium, in turn creating a secondary source. Additionally, some pathogens can survive on surfaces in the built environment even in the absence of other environmental media, which may warrant the inclusion of the built environment as a medium in a CEM. The built environment may also be incorporated in a CEM due to its role in **cross-media transfer** and exposure scenarios. Another category that can be treated as an environmental medium is biota (living organisms). Biota may include organisms that carry a BioCEC but are not adversely impacted by its survival, also known as reservoirs. Produce may be included as well, as a BioCEC transferred through **irrigation** or soil may live on its surface. More information about cross-media transfer and exposure scenarios can be found in **Cross-Media Transfer and Exposure Scenarios**.

3.1.4 The Epidemiologic Triangle and Key Variables

The **epidemiologic triangle** with vertices of pathogen, host, and environment is a model that emphasizes the interactions among a pathogen, a population susceptible to infection from the pathogen (host), and conditions favorable for exposure of the host to the pathogen (environment) (Figure 3-1). A CEM maps potential pathways of exposure from the pathogen source to the host via the environment, touching on each vertex of the epidemiologic triangle. A key variables evaluation is then used as a tool for prioritization and prediction for each step of the pathway (see the Key Variables for more information).

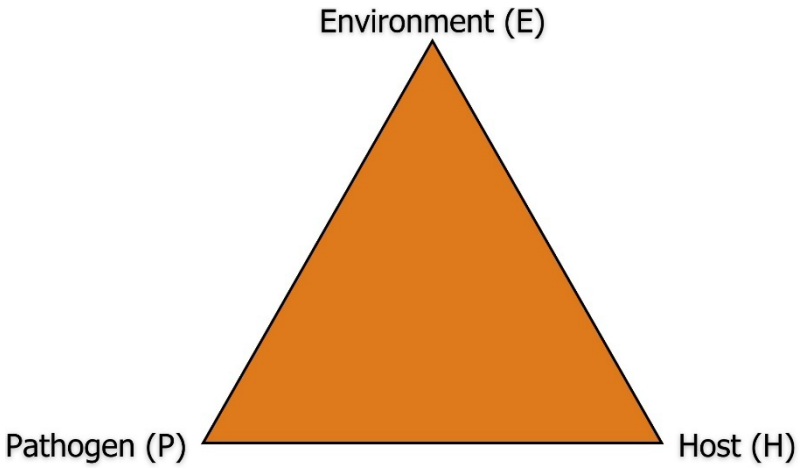

Figure 3-1. The epidemiologic triangle.

Source: Adapted from (Morabia 2013).

3.2 Building a Conceptual Exposure Model Using the Epidemiologic Triangle

3.2.1 Identification of Environment, Pathogen, and Host

CEM creation requires information regarding the environment, pathogen, and host. Identifying what is known about host populations, contaminated media, and the pathogen is helpful in establishing a scope, which may expand or shrink as data become available and knowledge gaps are addressed. Information used for identification may include data related to environmental sampling, host demographics, and pathogen screening. When applicable, vectors and the built environment should also be included in this stage of CEM development.

3.2.2 Source Media

A source is a contaminated medium that is primarily responsible for transmitting pathogens to exposure media or susceptible hosts. It may be difficult to identify the source of a BioCEC outbreak due to the ubiquity, movement, and lifespan of biological contaminants. Source identification may require temporal and spatial sampling. A source is not necessarily where the pathogen originated, but it may instead be a location where it thrives on a local scale. A source location and medium may also vary in pathogen loading on a temporal scale. Source examples and their extents include the following:

- A marsh that is used as a breeding ground for mosquitoes.

    - The source is periodic due to seasonal variations in water levels and migration of mosquitoes, with the spatial extent limited to the breeding grounds.

- A wildfire that aerosolizes fungal spores.
    - This source is a single event with the spatial extent limited to the wildfire area, or the area that contained the fungal spores.

- Conveyance pipes that erode and introduce contaminated soil into potable water.
    - The source is continuously releasing contaminants into the water, with the spatial extent limited to the damaged area(s) of the pipe.

- Sewage released from septic tanks or wastewater conveyance infrastructure.
    - The source is intermittent, as contamination may only result after weather conditions facilitate movement or directly after application or release, with the spatial extent limited to the area of application or release.

A BioCEC does not necessarily require source identification. A relatively harmless pathogen that is already present within a localized part of the environment may become a BioCEC due to changes within the environment, host population, or pathogen characteristics, which are further described in the **Key Variables**.

3.2.3 Cross-Media Transfer and Exposure Scenarios

Contaminants can travel within and between different environmental media, which is a process referred to as cross-media transfer. Cross-media transfer is the result of natural or engineered mechanisms. A few example mechanisms of cross-media transfer are listed below:

- **Aerosolization** — A contaminant in soil or water becomes airborne.
- **Deposition** — An airborne contaminant lands on a surface.
- **Leaching** — Contaminants within soil or waste are transported in flowing water and move into environmental media on the surface or subsurface.
- Irrigation — Surface water, groundwater, or treated wastewater are supplied to land or crops. Irrigation can facilitate leaching.
- **Intake** — Water is taken from the environment and used as drinking water or for industrial and agricultural operations without complete disinfection.

Not all cross-media transfer results in an exposure scenario, as contaminated environmental media may not have a mechanism to interact with a host. For instance, buried waste in subsurface soil may seep into groundwater that is never extracted for human use. A complete pathway requires the pathogen to contaminate an environmental medium that the host directly interacts with, which we define as an **exposure medium**. Many factors influence the ability of a medium to be an exposure medium, such as pathogen viability, hydrologic and hydrogeologic conditions, and physical barriers that make it inaccessible.

Once a pathogen contaminates an exposure medium, infection becomes a possibility. An exposure scenario is the mechanism by which the host interacts with an exposure medium. Some exposure scenarios are listed below:

- **Dermal exposure** — Contaminated environmental media touches the skin or mucosal membrane of an individual.
- **Inhalation** — An individual breathes in an airborne contaminant.
- **Ingestion** — A individual eats or drinks contaminated media.

The mechanisms of cross-media transfer and exposure scenarios listed in this section are not exhaustive. Professionals specializing in scenario-relevant fields — such as water resources, agriculture, engineering, and public health — may need to collaborate to ensure that a CEM is complete and appropriate in scope. More information on this topic can be found in Gerba's "Environmentally Transmitted Pathogens" (Gerba 2015).

3.2.4 General Conceptual Exposure Model

The general CEM (Figure 3-2) demonstrates which components of the epidemiologic triangle (environment [E], pathogen [P], and host [H]) are relevant to BioCEC movement from an environmental source all the way to an infected individual. The arrows demonstrate how a BioCEC is transferred between each component. Figure 3-6 also identifies regulatory applications, which are described further in General Conceptual Exposure Model with Regulatory Applications.

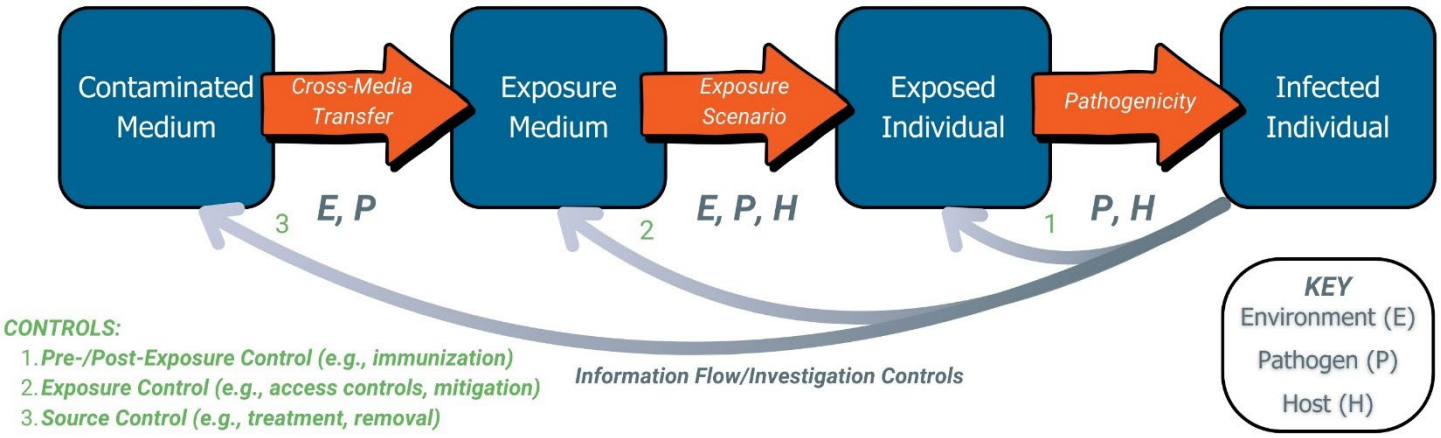

Figure 3-2. General conceptual exposure model.

The contaminated medium is the soil, water, waste, or air that contains a BioCEC. Cross-media transfer occurs when the pathogen travels to other media from the contaminated medium, generating an exposure medium. The exposure scenario is the mechanism by which an individual (human host) interacts with an exposure medium, becoming an exposed individual. To complete the pathway, the exposed individual must become infected or transmit the pathogen to another, creating an infected individual.

3.2.5 Iteration and Hypotheses

Sources, cross-media transfer, and exposure scenarios can be hypothesized based upon factors such as hydrogeology, pathogen characteristics, human host characteristics, and anthropogenic activity.

Environmental media and host populations can be sampled and analyzed to evaluate these hypotheses. The CEM should be updated as data become available and as conclusions are drawn. More information regarding cross-media transfer, exposure scenarios, and pathogenicity (e.g., virulence) can be found in the **Key Variables**. More information regarding environmental sampling can be found in the **Monitoring Programs / Resource Hub** and **Analytical Methods** sections.

3.3 Using a Conceptual Exposure Model

3.3.1 Applications

BioCEC can be challenging to address because disease outbreaks are often unanticipated, with regulators endeavoring to gather and evaluate information on the pathogen, host, and environment. A CEM is essential for developing an adequate BioCEC evaluation and response, as the information communicated is interdisciplinary, testable, and iterative.

Once a CEM that visualizes collective knowledge is developed, it becomes easier to identify knowledge and data gaps, refine the scope, create hypotheses, and formulate investigation and response strategies (see **Figure 3-2**). For instance, someone in the public health field may not have the expertise required to identify potential mechanisms of cross-media transfer, while an environmental professional may lack the knowledge needed to evaluate the viability of a pathogen in an environmental medium. After data are collected, a new CEM iteration can succinctly communicate updates without requiring expert analysis from each team member. Additionally, the CEM is adaptable, and can be modified to suit the needs of all stakeholders.

CEM applications may include the following:

- Identifying:
  - Knowledge or data gaps
  - Contaminated and potentially contaminated environmental media
  - Regulatory agencies with jurisdiction over different environmental media
  - Exposure scenarios
  - Susceptible host populations

- Planning:
  - Investigation strategies
  - Monitoring programs
  - Institutional controls and other interventions
  - Collaboration with all involved regulatory agencies

- Communicating:
  - Updates as new data are added
  - Information about the site to various audiences, including the public
  - With response team members with varying backgrounds

3.3.2 General Conceptual Exposure Model with Regulatory Applications

Figure 3-2 is a representation of how a CEM can be applied in every stage of pathogen transmission, from the contaminated medium to the infected individual; this application usually occurs once an outbreak is identified. Clinical identification of an infected individual is often where investigation and intervention begin. The gray arrows below the infected individual represent the regulatory responses of environmental and public health jurisdictions intended to disrupt pathogen transmission.

Three control strategies are shown in Figure 3-2 and are listed below. These may be proactive or reactive measures or both.

- Control 1. Pre-/post-exposure control impacts pathogenicity. This intends to reduce infection in potentially exposed individuals in a population, often through immunization or other prophylactic measures.
- Control 2. Exposure control impacts the exposure scenario. This intends to reduce human host exposure through **land-use controls**, such as physical barriers, signage, and restricted uses. Mitigation measures and environmental surveillance may be used in this stage as well.
- Control 3. Source control impacts the cross-media transfer at the source. This intends to remove the pathogen completely from the source or eliminate all potential exposures of another medium or host. It may also inhibit the pathogen from entering the exposure medium.

3.4 Conceptual Exposure Model Examples

This subsection presents two hypothetical CEMs as illustrative examples. The example CEMs are not meant to be all-encompassing but illustrate potential exposure pathways connecting one or more pathogenic sources to likely hosts via different cross-media transfer within the environment. Regulators should create their own CEM by following the steps described in Building a Conceptual Exposure Model Using the Epidemiologic Triangle — identifying the environment, pathogen, host, source media, cross-media transmission pathways, and exposure scenarios. If generic CEMs are used, a regulator may overlook factors specific to their BioCEC scenario. Each of the following CEMs use gray arrows to represent environmental transmission and red arrows to represent exposure.

3.4.1 Example 1: Biowaste Release to Soil / Land Application of Class B Biosolids

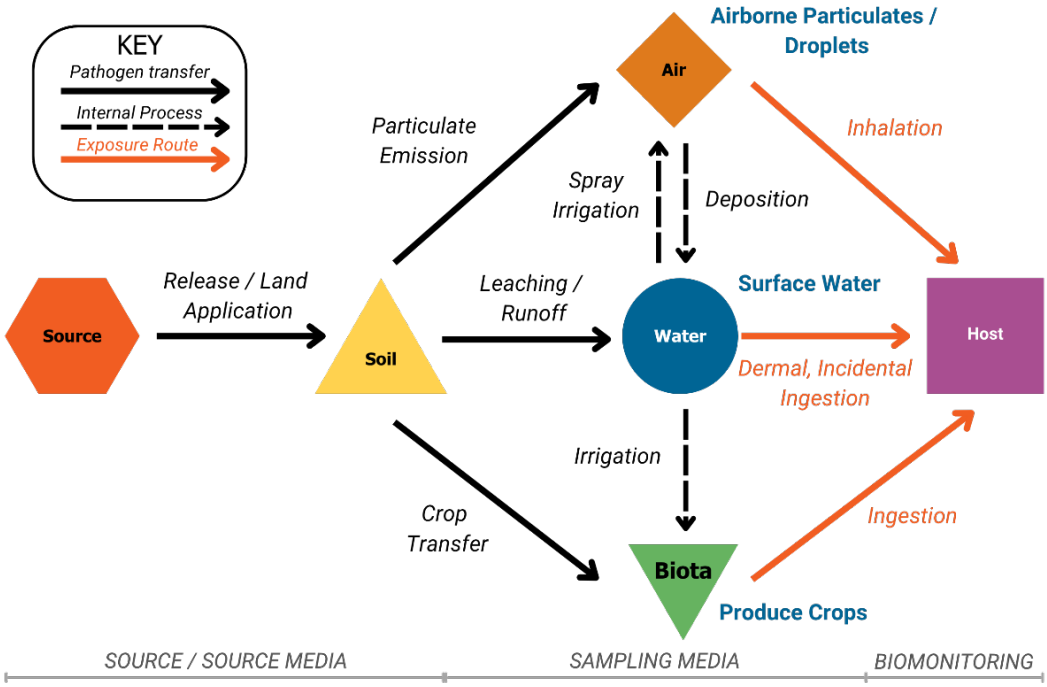

Figure 3-3. Biowaste release to soil / land application of Class B biosolids (Example 1).

Figure 3-3 is a CEM that demonstrates the introduction of a BioCEC through land application of animal biowaste (e.g., manure) or Class B biosolids, which unlike Class A biosolids is not thermally or chemically stabilized to eliminate pathogenic load in excess of background levels. The application of Class B biosolids to soils can lead to the potential exposure of a host via different cross-media transport mechanisms and exposure scenarios. The pathogenic source applied to the soil can travel to the different exposure media of air (via emission of mold and spores from soil disturbance, wind gusts, and wildfires), water resources such as groundwaters and surface waters (via leaching, runoff, and percolation), and biota (via transfer from soil or water to a crop). Incidentally, cross-media transfer can also occur between different exposure media, such as the particle deposition and resuspension illustrated between the air and water exposure media and the irrigation of produce crops using a contaminated water resource. It is important to note that every potentially complete exposure pathway from pathogenic source to host in Figure 3-3 can be its own stand-alone CEM controlled by different exposure scenarios (Figure 3-4).

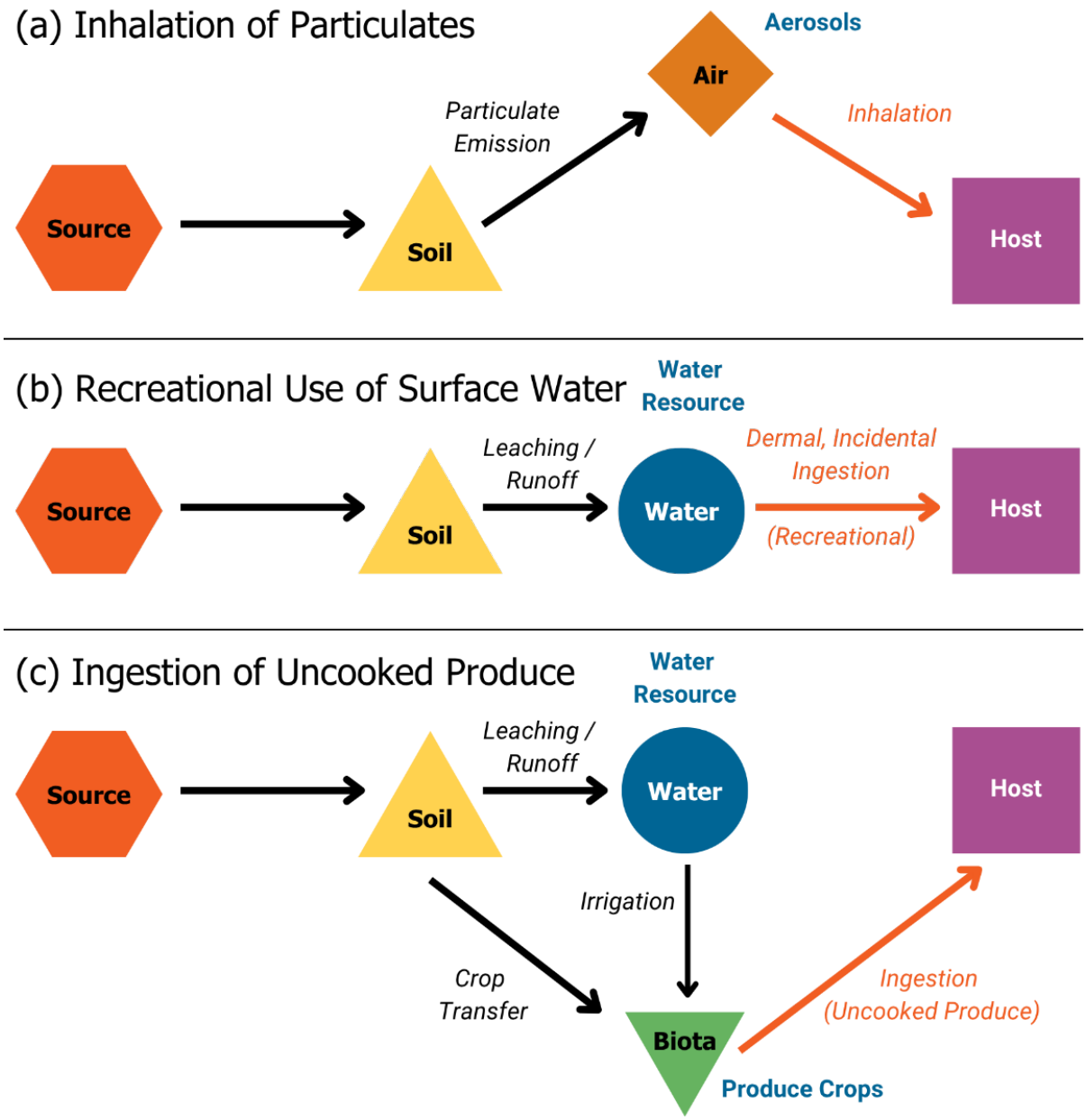

Figure 3-4. Individual exposure pathways within a conceptual exposure model (Example 1).

3.4.2 Example 2: Pathogenic Release to Potable Water Resource

Figure 3-5 is a CEM demonstrating how a BioCEC introduced into water through the release of a pathogenic source to a potable water resource can potentially make its way to a susceptible host through ingestion and dermal exposure to the potable water or to its resource. The example has two sources of pathogens: the release itself and the municipal wastewater generated from the consumption of potable water. Real-world scenarios of this example CEM could be septic tank discharge to groundwater, livestock bathing in surface water, or municipal wastewater release to a surface water body. It is notable that the individual exposure pathways within the example CEM represent their own CEMs, some of which are illustrated in Figure 3-6.

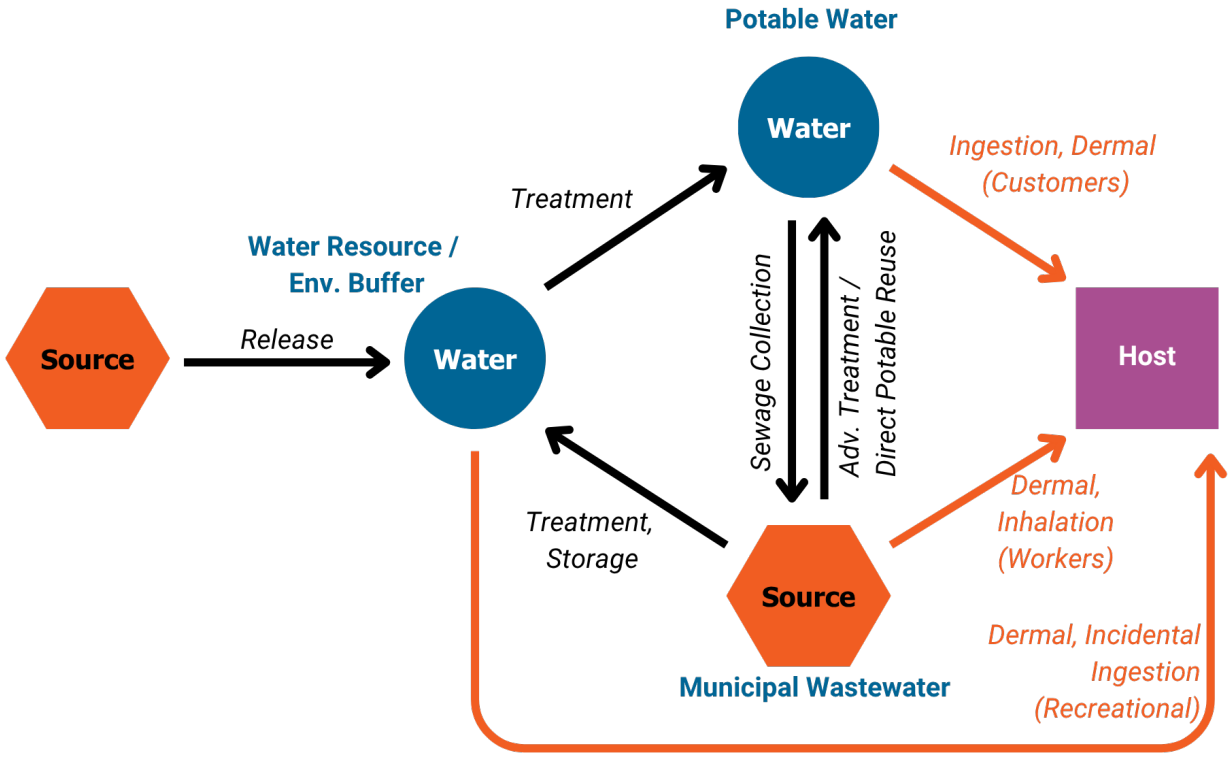

Figure 3-5. Pathogenic release to a potable water resource (Example 2).

Figure 3-6 (a), (b), and (c) all show failures of engineered barriers in the form of treatment and disinfection technologies. The three pathways, which represent drinking water treatment, direct potable reuse through advanced tertiary treatment technologies like reverse osmosis and advanced oxidation, and indirect potable reuse, can be subject to disinfection technology failures, resulting in residual pathogenic load in potable water. People drinking or bathing in such water can potentially become infected. Figure 3-6 (d) shows the recreational use scenario of a surface water body like a river or a lake where any releases to that water body can expose individuals using it for recreational purposes. A pathway shown on Figure 3-5 but not on Figure 3-6 is the dermal and inhalation exposure of aerosols by municipal wastewater treatment workers.

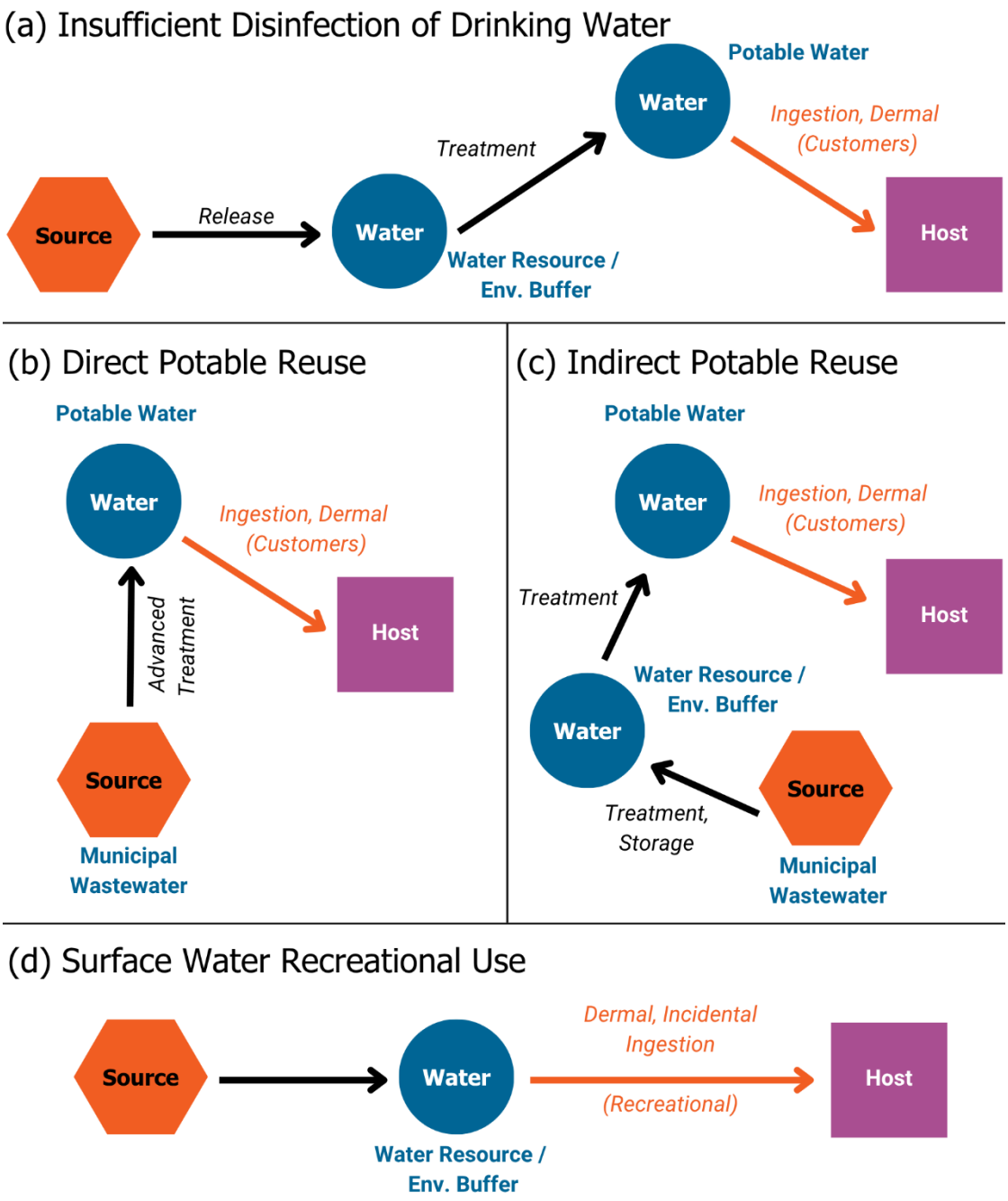

Figure 3-6. Individual exposure pathways within a conceptual exposure model (Example 2).

3.5 Case Study: Using a Conceptual Exposure Model to Address a 2018 *E. coli* Outbreak Linked to Romaine Lettuce

The case study found in Case Study: 2018 *Escherichia coli* Outbreak Linked to Romaine Lettuce provides an example of how a CEM was applied in a real-world scenario. The case study is associated with romaine lettuce grown from the Yuma region of Arizona that reportedly caused people in several states and Canada to become ill. Multiple state agencies, CDC, and the US Food and Drug Administration coordinated their investigations to understand the outbreak, including potential causes and

environmental transmission pathways, monitoring issues and approaches, stakeholder and community participation, current policies and procedures, and other factors that may have contributed to the outbreak. **Figure 7-8 in the Case Studies** section exemplifies how a CEM can incorporate both the confirmed and hypothesized presence of a BioCEC within environmental media, transmission pathways, and receptors. Although the source of the BioCEC in the case study was not identified, the information gained through the investigation can assist with monitoring transmission pathways, mitigating exposure, and reacting to future outbreaks.

4 KEY VARIABLES

4.1 Introduction

The emergence of previously unidentified **infectious diseases** and **pathogens** has stimulated much concern in the public health community. This has triggered an interest in the collection of pathogen-specific and epidemiological data to inform risk assessment, management, and mitigation practices. This can be a difficult task given the number of key variables that impact pathogen spread, persistence, transmission, success, and other factors, including changes in the demographics of exposed populations and immunocompetency. For example, pathogen emergence and reemergence in specific geographic locales encompasses everything from previously unidentified infectious agents entering the human population to established pathogens invading new populations and the evolution of drug resistance (Metcalf and Lessler 2017).

Traditional infectious diseases, together with emerging and reemerging infections, remain a major public health threat globally, and especially in low- and middle-income countries where resources are limited (Chen et al. 2023). Rapid urbanization, home insecurity, and deteriorating or already poor infrastructure has been changing the pattern of disease outbreaks, morbidity, and mortality. Land-use changes, such as agricultural expansion and deforestation, have changed the transmission of infectious disease directly through changing human contact with wildlife and **vectors**, but also indirectly through changes in biodiversity and pathogen spread due to increased travel, trade, and globalization. These changes may not only contribute to an increase in the transmission speed of outbreaks but also enlarge the scope of the transmission area (Wu et al. 2014).

In addition, the significant rise in antimicrobial resistance has compounded the challenge of infectious disease transmission prevention globally (WHO 2021). It was estimated that 1.27 million people died directly from antibiotic-resistant bacterial infections in 2019, and 4.95 million people died from illnesses in which bacterial antimicrobial resistance was implicated (Murray et al. 2022). Changes in water and solids recycling can also impact exposure to pathogens. Adoption of direct and indirect potable reuse has several benefits as drought-proof sources of drinking water, particularly in arid regions; however, there are concerns of increased exposure to **biological contaminants of emerging concern (BioCEC)** such as antibiotic-resistant bacteria and **opportunistic pathogens** (Garner et al. 2018).

Climatic hazards, such as sea-level rise, heatwaves, droughts, wildfires, floods, etc., can also impact disease transmission. Mora et al. (2022) reported that out of 375 infectious diseases documented globally, 58% will be aggravated by climatic hazards. The study also reported specific pathways through which climate change aggravation can occur:

1. Climatic hazards bringing pathogens closer to people (e.g., increases in vector and pathogen spread, spillover from viruses with viruses moving over larger areas following climatic hazard, storms causing wastewater overflows)

2. Climatic hazards bringing people closer to pathogens (e.g., water reclamation and reuse, increased recreational activities during heat waves)

3. Climatic hazards increasing the **virulence** of pathogens (e.g., increases in harmful algal blooms, rainfall increasing habitats for **vector-borne disease** transmission, heatwaves selecting for "heat-resistant" microorganisms)

4. People impaired by climatic hazards (e.g., stress and malnutrition that reduce immunity, people forced into unsafe conditions, damaged infrastructure)

Identifying key variables that predict the increased potential of environmental transmission of pathogens is crucial to proactively take measures to minimize the spread of BioCEC. The objective of this section is to characterize key variables that may be used to identify, evaluate, and prioritize BioCEC. In addition, resources that can be used for prioritization of BioCEC is summarized to help assess the risk of BioCEC and inform decisions. The section does not include person-to-person transmission and community spread of disease, since **communicable diseases** are not within the scope of this guidance.

Figure 4-1 shows the framework adapted the Centers for Disease Control and Prevention's (CDC's) One Health approach (CDC 2024), which illustrates a variety of macro-level factors, including the epidemiological triangle, that influence pathogen or BioCEC transmission and infectious disease risk. The epidemiological triangle (pathogen/BioCEC, **host**, and environment) in the center of the graphic is the framework used for the discussion of key variables in this section (see The Epidemiological Triangle).

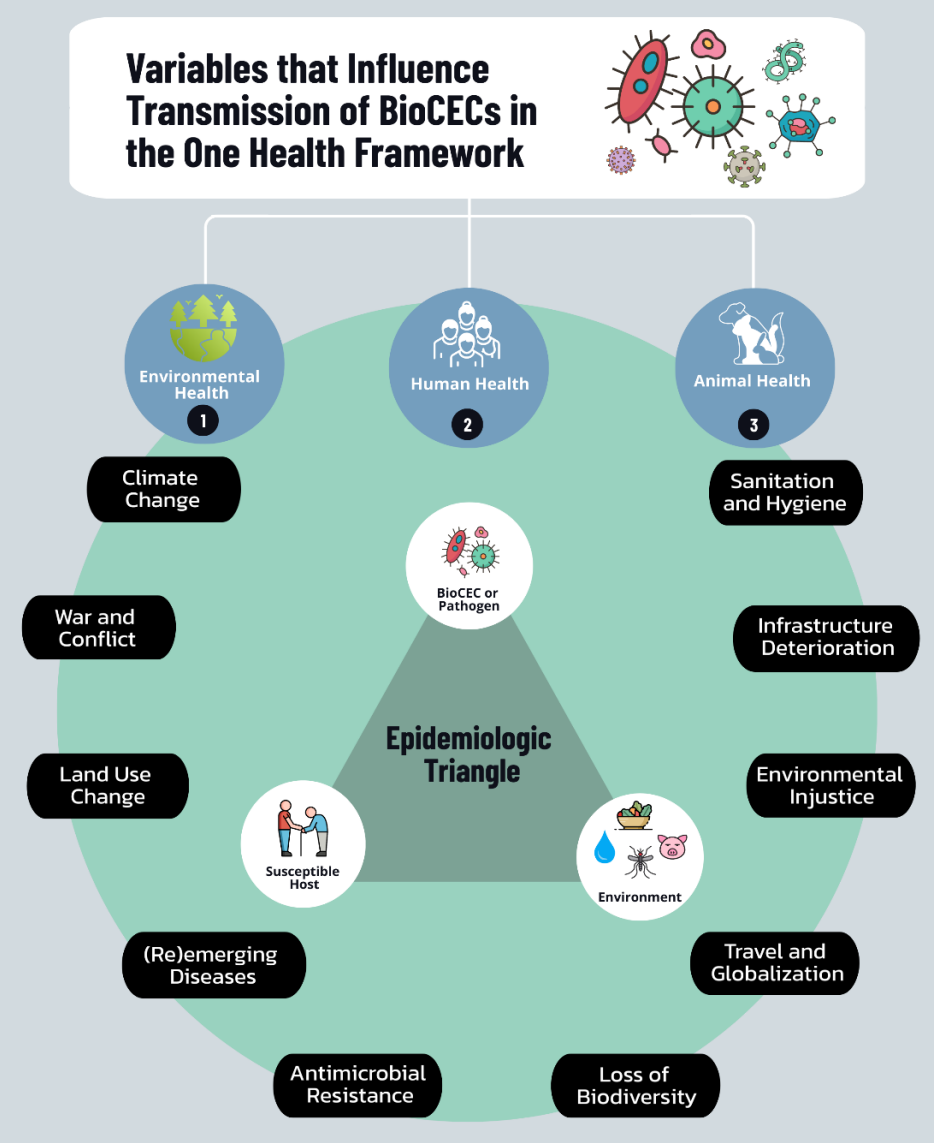

Figure 4-1. Variables that influence the transmission of biological contaminants of emerging concern in the One Health framework.

Source: Adapted from (CDC 2024).

The section is divided into six subsections. **The Epidemiological Triangle** describes the epidemiological triangle, which is the framework used for the discussion of key variables. This concept is used to describe the relationships and interactions among pathogen, host, and exposure through the environment to determine the impact of BioCEC on human health. These relationships and interactions for a specific BioCEC scenario, outbreak, or site of concern are better illustrated and defined using a site-specific **conceptual exposure model (CEM)**. As defined in the **Conceptual Exposure Model**, a CEM is a visual representation of a site, such as illustrations or block diagrams, that maps known and potential interactions among an environment, pathogen, and host. The relationships and interactions presented in the CEM inform the identification and evaluation of key variables that influence the presence and severity of a BioCEC scenario or outbreak. Likewise, the evaluation of key variables using newly available information provides feedback for changing the CEM. CEM development and examples are presented in the **Conceptual Exposure Model**.

In **Considerations for Assessing Risks from BioCEC**, we define and discuss key variables of each leg of the **epidemiologic triangle**. Key variables that fall under pathogen, host, and environment are identified and described to provide context for evaluation of risk of BioCEC.

In **Approaches to BioCEC Prioritization Strategies**, different prioritization schemes that are used to evaluate the risk of BioCEC are reviewed, including the World Health Organization's (WHO's) guideline, the US Environmental Protection Agency's (USEPA's) Contaminant Candidate List 5 (CCL5), and Health Canada. These prioritization approaches apply a set of defined criteria to determine whether there is high risk of BioCEC.

Tools for Prioritization includes a newly developed process description and flow chart developed by the Interstate Technology and Regulatory Council's BioCEC team to summarize tools used for prioritizing BioCEC. It also includes a discussion of the data and resources required for the different platforms for prioritization and the advantages and disadvantages of the different approaches.

Limitations and Knowledge Gap summarizes the limitations of the summary presented in this section and knowledge gaps in the assessment of key variables for BioCEC.

The **Case Studies** includes three case studies that demonstrate the application of quantitative microbial risk assessment (QMRA) to the evaluation of the risk of BioCEC. The case studies address the risk of salmonellosis from alternatively produced broiler meat, the risk of *Legionella* infections from two shower exposure models, and the use of QMRA for direct potable water reuse treatment targets in California.

4.2 The Epidemiological Triangle

Although numerous biological, social, environmental, and ecological factors contribute to the successful emergence of a human pathogenic disease (Metcalf and Lessler 2017), most public health professionals describe the risk of pathogen transmission as the result of three factors intersecting under the right circumstances: the pathogen, the host, and the environment. Although the concept of a disease triangle was conceived decades ago (Gäumann 1950), it took a while before it was widely applied in the context of public health and human disease transmission (John and Kompithra 2023).

The epidemiological triangle is a model used to describe the interactions among a pathogen, a population susceptible to infection from the pathogen (host), and conditions favorable for exposure of the host to the pathogen (environment). The epidemiologic triangle has been used to help explain the emergence and evolution of epidemics since it was first introduced in the 1920s (Morabia 2013). Although the epidemiological triangle, like the CEM, is useful to elucidate specific exposure scenarios (see the **Conceptual Exposure Model**) and key variables involved with BioCEC, it should be acknowledged that other models also help explain the variables involved with the potential spread of BioCEC at a higher, more holistic level. Examples include the One Health approach (see **Figure 4-1**), which connects animal,

human, and environmental health. One Health emphasizes collaboration among multiple disciplines to understand and combat the spread of disease and the eco-epidemiology triangle that explicitly addresses the concepts of ecology and transmission channels in host–pathogen–environment interactions (CDC 2024; John and Kompithra 2023). This multidisciplinary, and often interdisciplinary, approach to evaluating and responding to BioCEC is discussed in the **Process Guide**.

Understanding the key variables that affect the human health effects of pathogenic agents found in the environment requires consideration of the key factors in the epidemiological triangle (**Figure 4-2**; also see **Figure 4-1**) that influence the harmful health effects, including infectious disease development. The pathogenic agent (BioCEC) found in the environment (soil, air, water, waste) infect the host through various transmission (direct, vector-borne, or foodborne) and exposure pathways (**ingestion**, **inhalation**, dermal contact). Pathogens within the host can cause disease depending on variables that affect the disease development, such as host susceptibility and immune status and **pathogenicity** and virulence of the BioCEC/pathogen. These interactions are better illustrated using a CEM. See the **Conceptual Exposure Model** for uses and examples.

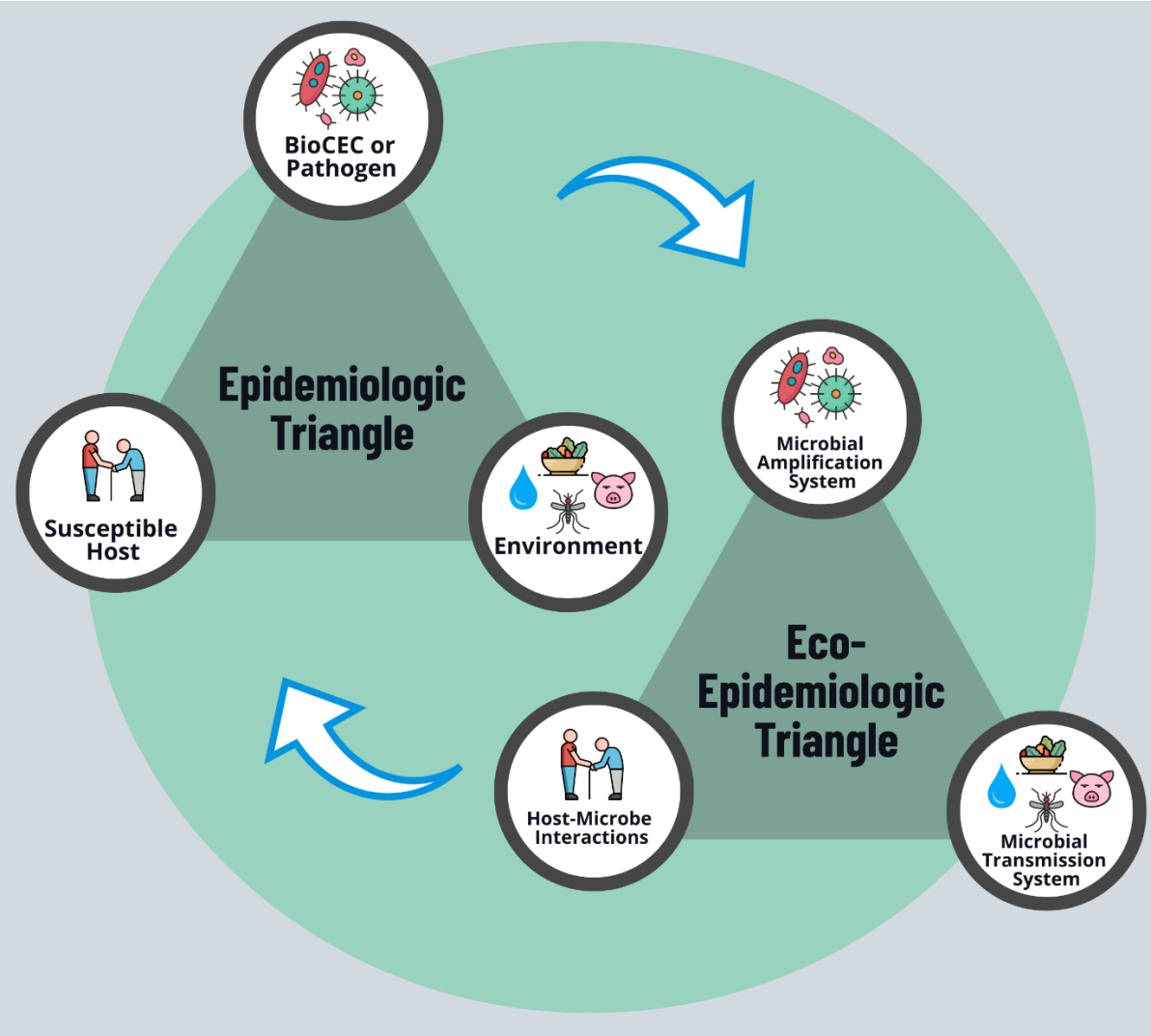

Figure 4-2. The epidemiologic triangle and the microbiological eco-epidemiologic triangle.

Source: Adapted from (CDC 2024).

It is noteworthy that the term 'environment' in the epidemiological triangle was used as a general term that applied to any and all factors and determinants of infectious diseases, including socioeconomic and demographic variables (John and Kompithra 2023). Some pathogens are directly transmitted from human to human or animal to human. In those cases, the infected host/reservoir/vector is actually the "environment" for the uninfected would-be host in the vicinity. Given the confusion this creates, several epidemiologists and authors have created the eco-epidemiological triangle (see **Figure 4-2**), which explains disease transmission in terms of microbial amplification systems, microbial transmission systems, and microbe-host pathogen interactions (John et al. 2024).

Due to the prevalence of concerns regarding the threat of anthroponotic and **zoonotic diseases**, this document includes some information on these types of diseases. Exposure to pathogens that are vector-borne requires an understanding of other environmental factors (e.g., humidity, rainfall, temperature) that can increase or change the vector population. This section will also cover vector-borne diseases specifically focusing on vectors (mosquitoes, ticks) that are affected by changes in the environment. Certain foodborne diseases will be discussed briefly in this section when the exposure pathway of concern is associated with pathogenic contaminants discovered in farm soil or water used for **irrigation**.

4.3 Considerations for Assessing Risks from BioCEC

4.3.1 Pathogens

The first consideration when assessing risk from a BioCEC is whether or not the organism is considered a pathogen. A pathogen is traditionally defined as an organism that can cause disease in a host, with the severity of the disease symptoms being referred to as virulence (Mara and Horan 2003). A host is an organism that harbors a pathogen or parasite. The outcome of the microbe-host relationship depends on the pathogenicity of the organism and the susceptibility of the host. Pathogens are taxonomically diverse and comprise a broad range of viruses and bacteria, as well as unicellular eukaryotes (e.g., amoeba, algae, protozoa) and multicellular eukaryotes (e.g., fungi) (Madigan et al. 2021). All organisms are susceptible to pathogens, including bacteria, which are targeted by specialized viruses called phages. More recently, there has been some debate among researchers that the original definition of pathogenicity is insufficient and that pathogens should be defined as organisms that can cause harm or damage to a host (Balloux and van Dorp 2017). There are several ways to categorize pathogens, including by the level of dependence on the host for survival (e.g., intra- vs. extracellular pathogens) or the likelihood of triggering illness (e.g., strict versus opportunistic pathogen). Pathogen classifications tend to overlap and are not mutually exclusive, but they are explained below to provide the reader with clarity on the differences. Some familiarity with pathogen properties will help create a standardized dialogue among microbiologists, epidemiologists, and public health professionals. It also helps explain risk characterization and mitigation strategies to reduce risk.

Vignette #1. Extracellular vs. Intracellular Pathogens

The distinction between extracellular and intracellular pathogens is increasingly muddled.

It is noteworthy that an increasing number of bacteria, which we had previously described as extracellular (e.g., *Pseudomonas aeruginosa*, *E. coli*, *Vibrio cholerae*, and *Acinetobacter baumannii*) have documented **strains** that exhibit facultative intracellular characteristics (Pirofski and Casadevall 2012). This has resulted in a debate around whether the capacity for intracellular survival may be more common than previously thought (Casadevall 2008). Given the major implications for human health and disease, as well as infection control and treatment, most public health professionals continue to use this classification system.

4.3.1.1 Intra- and Extracellular Pathogens

Classically, infectious agents have been classified as extracellular, facultative intracellular, and obligate intracellular pathogens (Leon-Sicairos et al. 2015). This has major implications for how the pathogen replicates and enters the host (i.e., how risk can be mitigated), whether immunity can be developed, and whether medication can be effective against the pathogen.

Vignette #2. Extracellular vs. Intracellular Pathogens

Distinguishing facultative and obligate intracellular parasites.

The classical division between facultative and obligate intracellular parasites has been based on the presence (in facultative intracellular bacteria) or absence (in obligate intracellular bacteria) of the capacity to multiply in a cell-free environment, which is evaluated using artificial bacteriological media (Silva and Silva Pestana 2013). Obligate intracellular bacteria cannot be grown in artificial media (agar plates/broths) in laboratories because they require viable eukaryotic **host** cells (e.g., cell culture, embryonated eggs, and susceptible animals). For example, *Chlamydia trachomatis* is an obligate intracellular bacterium. Chlamydial cells depend on the host cell for adenosine triphosphate and other intermediate molecule production because they are unable to carry out energy metabolism and lack many biosynthetic pathways.

Staphylococcus aureus, Pseudomonas aeruginosa, Streptococcus pyogenes and most *E. coli* **strains** are examples of extracellular pathogenic bacteria that need a "portal of entry" or "transmission route" to enter the host, and they cause a range of infections. These organisms usually have the capacity to survive and sometimes replicate in the environment outside of the host (Silva 2012), which has implications for the CEM, microbial transmission, and the amplification systems in the eco-epidemiological triangle.

Transmission routes to reach the site of injury or infection include water, air, wound, food, mechanical vectors, and fomite contact. Once arrived, these pathogens multiply in the host at extracellular sites or outside of cells (e.g., mucosal surfaces; interstitial spaces; or vascular, lymphatic, or body cavity fluids) (Silva and Silva Pestana 2013) to cause damage and use virulence mechanisms to evade the immune system, thus promoting extracellular multiplication (Leon-Sicairos et al. 2015).

Intracellular survival is considered an evolutionary strategy that decreases competition between the pathogen and other organisms and improves chances of avoiding predation by amoeba in the environment (i.e., survival inside predator amoeba similar to *Legionella and Campylobacter*) resulting in endosymbiotic existence within protozoa (Casadevall 2008). Intracellular pathogens must enter into host cells to survive and reproduce (e.g., **macrophages**, epithelial cells), which makes them especially relevant from a public health perspective and difficult to eradicate (Leon-Sicairos et al. 2015; Pirofski and Casadevall 2012; Silva 2012; Silva and Silva Pestana 2013). Moreover, once inside host cells, they use specific pathways to evade the adaptive immune response to survive and replicate within host cells, as they are not directly accessible to antibodies, and their persistence relies on evading cell-mediated immunity. An example of this is *Legionella pneumophila, which* prefers the intracellular environment of macrophages for growth and blocks host destructive enzymes inside cells.

Obligate intracellular bacteria include *Coxiella burnetii*, *Rickettsia* spp.; certain protozoa such as *Trypanosoma* spp., *Plasmodium,* and *Toxoplasma;* fungi such as *Pneumocystis jirovecii;* all viruses and many vector-borne pathogens (Leon-Sicairos et al. 2015; Silva 2012; Silva and Silva Pestana 2013). When intra-cellularity is transient, the organism is considered a facultative intracellular pathogen (e.g., *Francisella tularensis, Listeria monocytogenes, Salmonella typhi, Mycobacterium* spp.). These are capable of living and reproducing either inside or outside host cells, making their lifestyles more adaptive (Leon-Sicairos et al. 2015).

Vignette #3. Opportunistic Pathogens

What makes opportunistic pathogens different?

Berg et al. (2014) suggested that most **opportunistic pathogens** will have one or more of the following features:

They elicit antagonistic activity against other microorganisms.

They are versatile in terms of nutritional needs.

They are cultivable.

They are copiotrophs (i.e., organisms that thrive in environments with abundant nutrients, particularly carbon).

They are highly competitive.

They are able to form **biofilms**.

They can be hypermutators.

They often have antibiotic and toxin resistance.

The authors hypothesized that more species of opportunistic pathogens will emerge as superbugs in the future since most of these characteristics are **strain**-specific and acquired through horizontal gene transfer.

4.3.1.2 Strict and Opportunistic (or Facultative) Pathogens

Opportunistic or facultative pathogens are organisms for which the host is only one of the potential niches they can exploit to reproduce (Balloux and van Dorp 2017). They usually do not cause disease in healthy hosts and are primarily environmental bacteria, parasites, or fungi that can occasionally cause infection when the right conditions present themselves (Haas et al. 2014). This has implications for the susceptible population and controls of pathogen in the environment to mitigate risk**.** In addition, many of the characteristics that lead to the success of opportunistic pathogens in colonizing hosts were properties initially necessary for their survival in the natural habitat (e.g., enhanced resistance to phagocytosis, resistance to irradiation or disinfection) (de Hoog et al. 2024). An example is **biofilm** formation. Biofilm formation is a catalyst for persistence and resistance to antimicrobials and changing environmental conditions that would otherwise result in pathogen elimination (WHO and International Water Association 2009; Flemming and Wuertz 2019; Palmer et al. 2007; Singh et al. 2021). Meanwhile, obligate pathogens require a host to fulfill their life cycle (i.e., stages of development of the parasite or pathogen until it reaches its mature infectious stage).

All viruses are obligate pathogens since they require the host's cell for their reproduction. Obligate pathogens are found among bacteria, including the agents of tuberculosis and syphilis, as well as protozoans (such as those causing malaria) and macroparasites (Balloux and van Dorp 2017). Some obligate pathogens require multiple hosts to complete their life cycle. The definite host, which supports the adult form of the pathogen, is often a vertebrate, and the intermediate host (referred to as a vector) is generally an arthropod or a mollusk. This alternation of vertebrate and invertebrate hosts is found in viruses (for example the Zika virus), bacteria (for example Lyme disease), and protozoa (malaria). Trematodes (parasitic flatworms or platyhelminths) go even further and require two or more intermediate hosts (Roberts et al. 2012).

When BioCEC require intermediate hosts, understanding all the components of the life cycle helps in the evaluation of infection control measures. For example, knowing that swimmer's itch (or cercarial dermatitis) requires a life cycle stage in snails and then in birds allows public health officials

Vignette #4. Biofilms

How relevant are biofilms to CEM development and risk assessment?

Biofilms are complex communities of attached microorganisms that are bound to biotic or abiotic surfaces by polysaccharides, proteins, and nucleic acids. This slimy matrix surrounding the cells is called an extracellular polymeric substance, and it creates a barrier that reduces susceptibility to environmental changes, disinfectants, and antibiotics. The extracellular polymeric substance also allows for concentration of nutrients, protection from phagocytosis and predation, extracellular enzyme usage by cells that cannot produce those enzymes, and increased horizontal gene transfer and retention of mobile genetic elements. Biofilm development leads to the formation of complex structures important for disease development, infection control, and engineering applications. Bacteria are far more likely to be found in biofilms in the natural environment than in planktonic (free- living) form.

to implement the right tools for monitoring, controlling, and eradicating the cause. It also helps in the development of an accurate CEM as seen in the **Conceptual Exposure Model**.

Vignette #5. Host Microbe Interactions

How specific is the site of infection within the host?

The **host** contains a diverse range of niches that **pathogens** can colonize and inhabit, since each region within the host is physically and chemically distinct. The microbiomes of skin, the respiratory tract, and the gastrointestinal tract are highly diverse. The highly oxygenated environment of the lungs favors obligate aerobes, such as *Mycobacterium tuberculosis*. The dry nature of skin selects for desiccation-resistant *Staphylococcus aureus*. Meanwhile, the anoxic environment of the large intestines selects for obligate anaerobic bacteria, such as *Bacteroides* and *Clostridium* (Madigan et al. 2021). More detail on these processes are provided in supplementary material. The following sections on host-microbe interactions are adapted from Brock's *Microbiology* (Madigan et al. 2021). Regardless of the final site of infection, the pathogen has to gain access to and infect the host tissue. This includes four distinct stages: entry, survival, replication, and exit from the host cell. These stages are shown in **Figure 4-3**, along with other bacterial pathogen characteristics (Silva and Silva Pestana 2013).

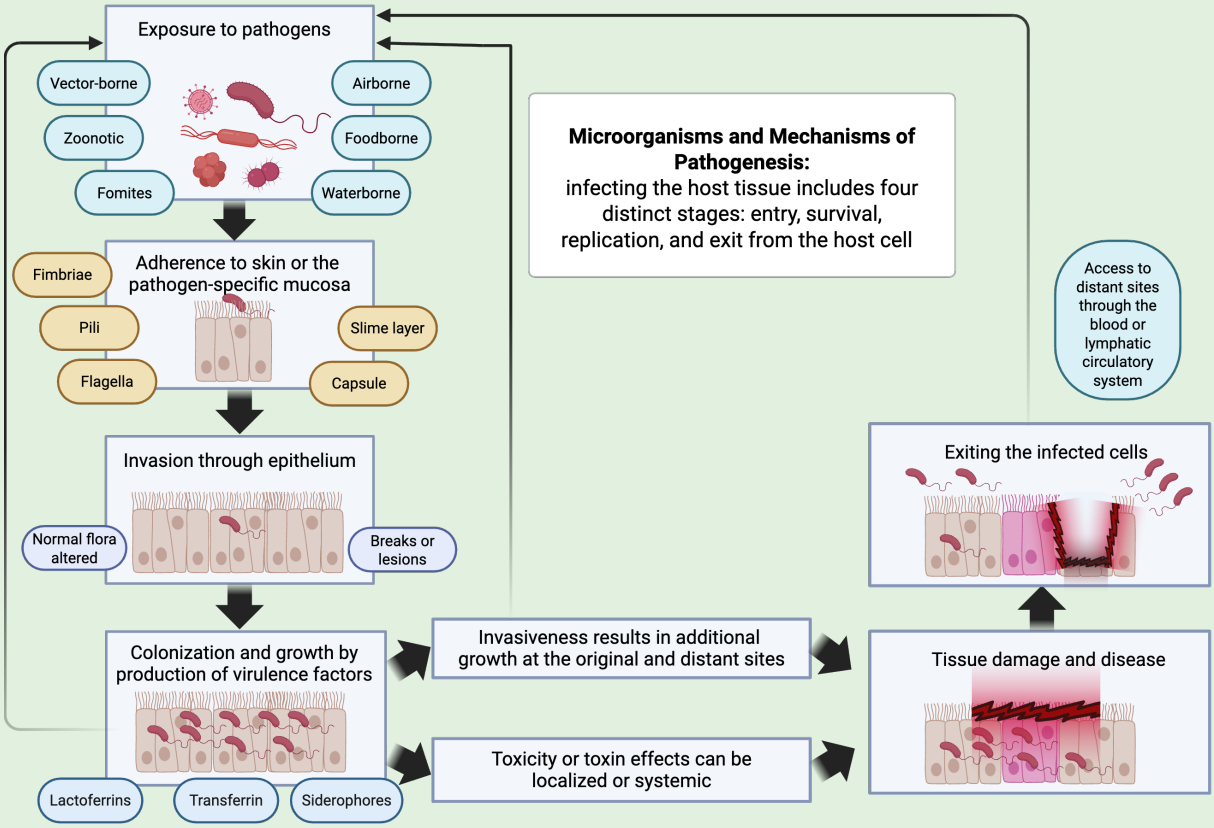

Figure 4-3. Mechanisms of pathogenesis.

Source: Adapted from (Madigan et al. 2021).

4.3.1.3 Infective Dose

The ability of an infectious disease to cause illness depends on the concentration of the microorganism in the environment the host is exposed to, the infective dose of the pathogen, and the virulence of the

pathogen (Ekdahl et al. 2005). It is commonly believed that the infective dose refers to a minimum threshold value above which infection occurs. In reality, the infective dose characterizes the *probability of infection* by providing the dose of pathogens above which the probability of infection exceeds a certain value.

The probability of illness to develop from an infection depends upon the degree of host damage (which can be influenced by age/life stage, preexisting illnesses, and factors that may make the host vulnerable or susceptible to damage) and whether this is sufficient to result in clinical symptoms (Bridle 2021; Madigan et al. 2021).

Overall, studies suggest that different groups of pathogens have different infective doses and levels of persistence. An example is provided in Table 4-1. The WHO considers the relative infectivity as low if the infective dose is greater than 10^4 pathogens, moderate for doses between 10^2 and 10^4, and high for doses between 1 and 10^2. There is no indication in the WHO list of what probability of infection this infective dose represents (Bridle 2021).

Vignette #6. Infective Dose and Virulence

How do we know what the infective dose of a pathogens is?

The infective dose can be estimated by the use of human challenge studies, outbreaks or 'natural experiments,' or a combination of both (Teunis 2022). Human challenge studies, or dose–response experiments, with healthy adult volunteers can be used to determine the probability of infection at different dose levels. Although these studies are typically regarded as the "gold standard," for determination of a dose-response assessment, they are ethically restricted to less virulent pathogenic **strains** and to healthy adult populations. Additionally, these studies often have limited sample sizes. Alternatively, the use of outbreak investigation data is helpful for mimicking "real-world" conditions. Outbreaks may, however, introduce bias by selecting for more susceptible **hosts**.

Humans can acquire infectious diseases even through exposure to very low levels of infectious particles. For example, an infectious dose of influenza A to humans is very low and is believed to be acquired through airborne and droplet means, whereas the infectious dose for *Francisella tularensis* is reported to be a single organism. Only a few cells of *Mycobacterium tuberculosis* are required to overcome normal lung clearance and inactivation mechanisms in a susceptible host (Cole and Cook 1998). The dose required for *Salmonella* spp. is influenced by the nature and physiological status of the strain, the matrix in which the strain is ingested, and the status of the potential host. Although the "typical" infectious dose is considered to be about 10^6–10^8 colony-forming units, epidemiological outbreak data suggest that the infectious dose can be substantially less, as little as a few cells (Cox and Pavic 2014).

Table 4-1. General characteristics of enteric pathogens and their impact on causing infections through water reuse.

Pathogen Type	Persistence in the Environment	Minimum Infective Dose	Immunity	Life Cycle Development in the Environment	Opportunistic or Strict Pathogen
Viruses	Medium	Low	Long	No	Strict
Bacteria	Short to medium	Medium–high	Short to medium	No	Both
Protozoa	Medium	Low–medium	None/little	No	Both
Helminths	Long	Low	None/little	Yes	Both

Source: Adapted from (Mara and Horan 2003).

Vignette #7. Infective Dose and Virulence

How do bacterial pathogens enter and colonize host cells?

In most cases reaching the **hosts** site of infection requires that the **pathogen** penetrate surfaces that would usually be considered barriers to infection (e.g., skin, mucous membranes, intestinal epithelium). Most microbial infections begin at breaks in the skin (e.g., cuts, wounds) or possibly lesions in the mucous membranes of the respiratory, digestive, or genito-urinary tract. Epithelial tissue attachment requires macromolecular interactions on the surfaces of both the pathogen and the host cell (e.g., binding to specific cell surface proteins). The attachment tends to be fairly selective, and pathogens do not adhere to all epithelial cells equally. In addition, adhesion strength can be fairly host specific, so that attachment in the correct final host is stronger, influencing host range where successful colonization and infection can occur. Attachment to host cells can be accomplished through **slime layers**, **capsules**, fimbriae, and pili. In addition, some bacteria produce enzymes and toxins to initiate **pathogenicity**.

Some macromolecules responsible for bacterial attachment are not covalently attached to the bacteria (e.g., polysaccharides, proteins, protein-carbohydrate mixtures) but secreted by bacteria. This loose network of polymeric fibers extending outward from the cell is called a slime layer. A polymer coat consisting of a dense, well-defined layer surrounding the cell is called a capsule. These structures are not just important for attachment to the host but may also influence interactions with surfaces and attachment to other bacteria.

Fimbriae and pili are bacterial cell surface structures that may also influence cell surface protein structures critical to the attachment process. Among the most detailed descriptions of fimbriae are the Type I fimbriae of enteric bacteria, which are uniformly distributed on the cell surface (e.g., *Escherichia*, *Klebsiella*, *Salmonella*, *Shigella*). Pili are typically longer than fimbriae, fewer in number, and are found on the surface of the cell. Both pili and fimbriae function by binding to host cell surface glycoproteins, initiating attachment. Flagella are also known to improve attachment to host cells.

To accomplish invasion, pathogens must penetrate the epithelium to initiate pathogenicity. Toxin-producing bacteria are the exception to this rule, and do not need to invade the host cell. Growth can occur after entry through a lesion in the mucosal membrane or on the surface if the microflora of the epithelium is altered enough. Pathogens then colonize the tissue and potentially grow in numbers or expand to more distant sites within the host through the blood or lymphatic circulation systems.

Enzymes can promote spreading of the organism by breaking down molecules that act like physical barriers to spread (e.g., streptococci and staphylococci produce hyaluronidase, which breaks down the polysaccharide hyaluronic acid that acts like an extracellular cement between animal cells).

Enzymes (e.g., proteases, lipases, nucleases) can break down intracellular host cell enzymes and disrupt basic functions. Some enzymes (e.g., collagenase produced by clostridia) disrupt the collagen network supporting host tissue, thereby allowing spread through the body.

Some pathogens produce enzymes that produce fibrin clots (e.g., coagulase produced by *S. aureus* cells) at the site of infection to localize and protect the organism. Coagulase also helps deposit fibrin inside the *S. aureus* cells to protect it from host cell attacks.

4.3.1.4 Virulence

Virulence is the degree of severity or harmfulness of a disease caused by a pathogen (Madigan et al., 2021). It is in part enabled by the organism's **toxicity** and invasiveness. Virulence factors are bacterial components or molecules that enable a pathogen to establish itself, evade the immune system, and cause disease by enhancing its ability to attach to host cells, damage tissues, and/or produce toxins.

Virulence is estimated from experimental studies of the **lethal dose$_{50}$ (LD_{50})**. The LD_{50} is the single dose of an organism, compound, or substance that is expected to kill 50% of a group of test animals in a

laboratory setting. The LD_{50} dose is usually expressed as milligrams or grams of material per kilogram of animal body weight (mg/kg or g/kg).

For highly virulent pathogens, the dose it takes to kill 50% of the population may be only slighter lower than the dose it takes to kill 100%, making exposure reduction crucial. Only a few cells of the highly virulent *Streptococcus pneumoniae* are required to establish a fatal infection and kill all animals in a test population. In comparison, the LD_{50} of the moderately virulent *Salmonella typhimurium* is much higher, and the number of cells of *S. typhimurium* needed to kill 100% of the population is almost 100 times higher than the organism's LD_{50} (Madigan et al., 2021).

Vignette #8. Determining LD_{50}

How do toxicologists determine an LD_{50}?

Toxicologists can use many kinds of animals, but most often testing is done with rats and mice. It is usually expressed as the amount of chemical administered (e.g., milligrams) per 100 grams (for smaller animals) or per kilogram body weight of the test animal (for larger test subjects). An **LD_{50}** can be determined for dermal contact (applied to the skin) and oral (given by mouth) administration methods (Canadian Center for Occupational Health and Safety 2025). This is different from the Lethal $Concentration_{50}$, which specifically refers to the concentration of a chemical in air or water.

Vignette #9. Strains: Phenotypes Vs. Genotypes

How are phenotype and genotype different?

Genotype is determined solely using genetic information dictating specific traits, whereas microbial **phenotype** specifically looks at the expressed (i.e., visible) traits of organisms, which are influenced both by both genetic determinants and environmental factors (Wiedmann 2019). Assessing **strain** virulence through "observed" variability at the phenotype level provides helpful information to assess other relevant factors, including stress resistance, distribution, **virulence**, and epidemiology of the pathogenic organism. Moreover, with particular reference to foodborne **pathogens**, it has been acknowledged that intra-species variability may have an important impact on the accuracy of microbiological risk assessment (Lianou et al. 2020). It is important to know that virulent strains kept in laboratory cultures, as opposed to isolation from diseased animals, undergo **attenuation**, which is a decrease or loss of virulence. Non-virulent or weakly virulent mutants are subject to faster growth and thus end up being selectively favored and outcompeting highly pathogenic strains. Attenuated strains are often used for vaccine production.

Essential bacterial genes orchestrate core biological processes and represent the targets of nearly all antibacterial drugs (Bosch et al. 2021). While genotyping determines the differences in the genetic makeup of the pathogen by examining the individual organism's DNA or RNA sequences using molecular tools, having a gene does not necessitate its expression or activity. Genes can be up- or downregulated. Partial inhibition of some essential genes results in decreased bacterial fitness, whereas other essential genes can tolerate substantial inhibition with little effect on bacterial fitness. This expression–fitness relationship is defined as gene vulnerability. Gene vulnerability relates the magnitude of gene expression inhibition with the resulting decrease in organismal fitness, thus describing gene essentiality as a continuous trait (Bosch et al. 2021).

When referring to virulence, the term strain variability is often used to describe the inherent differences among identically treated strains of the same microbial species. Historically, strain has meant a microbial isolate, although the definition is not well-suited to microbial community studies. Now, the term is used to refer to a specific microbial genome or collection of clonally identical cells (i.e., a **genotype**); one or more colonies (believed to be) derived from the same progenitor cell; or most often, in practice, a collection of cells or genomes within a relatively small range of phylogenetic variation (i.e., a very narrow subspecies **clade**) (Yan et al. 2020). Given this definition, to differentiate pathogens beyond the species level (i.e., strain typing or subtyping), genotypic or phenotypic characteristics are used (Lianou et al. 2020).

Extracellular **capsules**, **slime layers**, cell walls, envelopes, fimbriae, and pili are all integral to a pathogen's ability to produce infection, act as virulence factors, and are somewhat distinct from other intra and extracellular components that function solely as virulence factors with similar modes of action — for example, **exotoxins** (e.g., enterotoxins) and **endotoxins** enzymes. A broad range of toxins that pathogens can produce are considered virulence factors. Toxins are divided into two categories: exotoxins (e.g., enterotoxins) and endotoxins.

4.3.2 Environment

The environment is a key factor in understanding the spread and potential adverse effects of BioCEC. An environment can be characterized by scale, time, and geographic or biological location. A geographic location and the population living within it have a reciprocal relationship. Socioeconomic factors describe a population's income, occupation, education, living conditions, opportunity, and resources while geographic factors describe a location's landforms, natural resources, climate, and built environment. Both geographic location and socioeconomic factors play a large role in a BioCEC event by impacting factors such as population distribution, urbanization, behavior, travel of people and goods, industry, and extent of regulatory involvement. Biofilms that occur in engineered environments such as wastewater treatment plants, water reuse systems, and distribution infrastructure could differ from biofilms and pathogen occurrence in natural environments in non-developed or non-urbanized settings. The proper control and operation of engineered environments limits human exposure to pathogens. Environment is a broad term that can be used to describe the human body as an environment or a geographical region, such as an estuary or the Southeastern United States. Human environments are characterized by differences in cultural, physical, and biological conditions. Rapid growth in a human population or behavioral patterns such as frequent travel over long distances can affect the prevalence and potential of exposure to pathogens. A thorough consideration of the environment where a pathogen and host might interact helps to assess the risk of illness (Craun et al. 2010; WHO 2025).

It is important to consider environmental variables that affect pathogens and potential hosts as well as vector populations. Some pathogens survive in multiple media, although they might only flourish and multiply in one medium. Similarly, certain pathogens thrive within a particular temperature range, although they might survive outside their preferred conditions. An environment that is conducive to survival and multiplication of a pathogen will lend itself to generating a sufficient concentration to infect a host. Other considerations include antibiotic-resistance genes that can be a cofactor for pathogen virulence and epigenetics, which result in changes to how genes work, thus potentially impacting the vulnerability of a host to a pathogen. Stressors other than potential BioCEC might be present, such as chemical contamination or physical stressors such as heat or even psychological stressors associated with events like natural disasters. Although waterborne transmission of disease is well documented, pathogens can be transmitted by other media including soil and air (Craun et al. 2010).

The **WHO's website** provides information about how environment influences the spread of diseases. The prevalence of pathogens can increase depending upon local climate conditions. Weather events such as flooding can lead to spillage from sewage systems or damage to systems that provide clean drinking water.

Environmental factors vary widely from place to place and with time. The following list provides examples of environmental factors that can affect risk of exposure and infection from BioCEC:

- Anthropogenic activity
    - Waste disposal or reuse (impoundments, landfills, biosolid applications)
        - Waste release
        - Solid waste landfills
        - Sewage/septic tank (domestic waste)
        - Medical waste, animal waste
        - Land disposal of fecal-contaminated solid waste (diapers)
    - Agriculture (crops and livestock)
        - Produce farming
        - Biosolids (Class B) application
        - Recycled water (non-potable reuse and potable reuse)

        - Animal feeding operations or agricultural animal production

    - Infrastructure and urban operations
        - Water supply and treatment
        - Cooling towers, plumbing, etc.
        - Treatment: for example, water disinfection, removal/inactivation, failures in treatment barriers

- Geography and weather
    - Seasonality
    - Events that depart from historical norms
    - Events exacerbated by climate change:
        - Wildfires
        - Seawater intrusion into coastal aquifers and water distribution lines
        - Algal blooms
        - Reemergence of microbes from permafrost
        - Multiple factors that affect pathogen survival in the environment: temperature, moisture, pH, organic matter, native microbial flora

- Vector characteristics
    - Characteristics particular to thriving in the environment
    - Ability to infect human receptors
    - Factors that affect vector population density
    - Reservoir and intermediate hosts
    - Transported as aerosols/airborne droplets

As the list above implies, environmental factors can be divided into multiple categories. The WHO summarizes the most important environmental factors as water supply, sanitation facilities, food, and climate. When there is disruption associated with these environmental variables, the potential for risk of exposure and infection can increase.

4.3.3 Host

In this subsection, the host refers to a human that is exposed to or harbors pathogenic agents found in the environment. A host can manifest the symptoms and health effects or show no symptoms (carrier or subclinical infection). The observation of health effects may also depend on the incubation period (from time of exposure until symptoms appear) or duration of the clinical spectrum of the disease. Transmission of pathogens from the **environmental media** (e.g., waterborne, airborne, soil) to the human host will be the focus of this section. Information on human host as a **source** of emerging BioCEC, such as human-to-human direct transmission, will not be covered in this section; information on infectious diseases due to human-to-human transmissions (e.g., influenzas) can be found on the CDC (2025) and WHO (2025) websites. This section will focus on host factors that increase exposure to pathogenic agents in the environment. Factors that can affect host response to exposure and infection are discussed in the **Process Guide**, including websites that can serve as information sources, such as those representing US federal and state health departments, international countries, and the WHO.

Categories of key variables that relate to the host in the host–environment–pathogen interactions are listed below:

1. Variables that allow or enhance exposure of the host to pathogens
2. Variables that increase the likelihood of infection or affect the health outcome of the infected host
3. Variables that reduce or eliminate the likelihood of pathogen contamination of the environment

The CEMs shown in the **Conceptual Exposure Model** illustrate several ways of transmitting pathogens found in the environment (water, soil, and air) and the exposure scenarios through which the pathogens enter the host. See the **Conceptual Exposure Model** for more details.

4.3.3.1 Variables that Affect Exposure to Pathogens

Pathogens can be transported through environmental media from various sources (e.g., biowaste released to surface water, biosolids land application) and infect the host. The exposure may be a result of host behavior patterns that affect exposure, such as those listed below:

- Inadequate hygiene practices (e.g., hand washing)
- Hand to mouth behavior
- Recreational or occupational use of unsafe waters
- Inadequate cover or protection from mosquitoes or ticks
- Recreational or occupational use of places declared to have high vector population density
- Presence of asymptomatic infected host or carriers (subclinical or no overt disease)
- Prevalence of the pathogen in the community
- Lack of awareness and information on unsafe environmental conditions (e.g., poor water quality)
- Lack of community involvement (or lack of individual awareness) with health department activities that inform on preventing or reducing exposures to pathogens in identified environmental media (soil and water) and contaminated plants

Many environmental pathogens can also be transmitted through non-environmental exposures, such as person-to-person and foodborne transmission. For example, norovirus can be transmitted via foodborne, person-to-person, or fomite exposure, as well as via water and possibly soil. The overall prevalence of the pathogen across various media can affect the population burden of infection.

4.3.3.2 Variables that Affect Health Outcomes

Once exposure occurs, the likelihood of infection or severe infection and the subsequent appearance of clinical symptoms or health effects may depend on the host characteristics such as the following:

- Sensitive or vulnerable subpopulations / life stage (e.g., children, pregnant individuals, the elderly)

- Presence of co-morbidities (e.g., cardiovascular disease, cancer, diabetes, chronic infections, inadequate nutrition, or chronic stress)

- Immunological status (lack of prior exposures, immunocompromised patients, unvaccinated individuals)

- Susceptibility due to genetic or predisposing factors

The health effects resulting from pathogenic infections depend on the variability of the characteristics between individuals and among populations. Therefore, unacceptable health outcomes are better addressed by preventing or reducing exposure to pathogens in contaminated media. Characteristics of sensitive subpopulations can also influence both the exposure to pathogens as well as the severity of the health outcome. For example, children usually have poorer hygiene, which increases their exposure to pathogens, and they can experience more severe health effects following infection. Specifically, hemolytic uremic syndrome following *E. coli* 0157-H7 infection is a serious kidney condition that can result in severe illness and death and is much more common among children under five years old. Young children (especially infants and newborns) also may have less-developed immune systems and lack preexisting immunity to some infections. Legionellosis is much more common (and health effects are much more serious) among elderly individuals and those with chronic lung conditions.

4.3.3.3 Variables that Reduce or Eliminate Host Exposure to a Contaminated Environment

Reducing potential exposures of the host to environments that are known or likely to contain pathogens is a proactive intervention approach. Strategies include the following:

- State and local surveillance programs that address potential waterborne, soilborne, or vector-borne diseases that are occurring in specific localities, are likely to occur in certain areas when environmental conditions change, or may occur if migration from nearby endemic states occurs.

- Monitoring/surveillance programs, including the monitoring of areas with infected human cases, and having open communication with the public about control strategies through educational materials, guidance materials, press releases, and public meetings/discussions with experts.

- Vector control programs by local health departments.

- Public health guidance and outreach for specific pathogens of local concern.

- Provision of specialized guidance to immunocompromised and other susceptible individuals.

- Involving the community and health practitioners to report cases to the local health department.

- Mobilizing the community to seek ways to prevent exposure and eliminate conditions that allow vectors to multiply.

4.3.4 Host–Pathogen–Environment Interactions

Hosts provide a favorable environment for the growth and reproduction of pathogens by providing organic and inorganic nutrient supplies, growth factors, and stable environmental conditions (e.g., pH, osmotic pressure, temperature). Regardless of the final site of infection, the pathogen has to gain access to and infect the host tissue, and this includes four distinct stages: entry, survival, replication, and exit from the host cell (Silva and Silva Pestana 2013).

Understanding how a pathogen reaches its host can be extremely important to breaking the chain of infectious disease transmission. To be successfully transmitted to the next susceptible host, the pathogen must have some **environmental persistence** and be able to survive outside the host without loss of viability and infectivity.

Host-dependent pathogens gradually lose viability and their ability to infect after they are shed from a host. Pathogens with low persistence are unlikely to be spread through the environment, since they would be nonviable or noninfectious by the time they reach a new host (Bridle 2021). An example of this is SARS-CoV-2, which is an enveloped virus and was not spread through water or wastewater. In addition, some pathogens may be capable of growth in water, especially when the water is warm and high concentrations of biodegradable organic carbon are available (e.g., *Legionella, Vibrio cholerae, Naegleria fowleri*), which can occur in some surface waters or water distribution systems. Other pathogens (e.g., norovirus or *Cryptosporidium*) are unable to multiply in water but are robust enough to survive for a considerable length of time, thus improving persistence and likelihood of transmission (Bridle 2021).

A recent review by Hopkins et al. (2022) highlighted that 75% of the world's most burdensome 150 infectious diseases are environmentally transmitted. The review reported that nearly all infectious organisms were "environmentally mediated" to some degree, meaning that they spend time in reservoirs and can be transmitted from those reservoirs to human hosts. As a result, infection control and prevention can be primarily controlled through environmental interventions (e.g., control of host vectors or water sanitation), whereas few environmentally transmitted diseases (14%) were primarily controlled by integrated methods (i.e., combining medical and environmental interventions) (Hopkins et al. 2022).

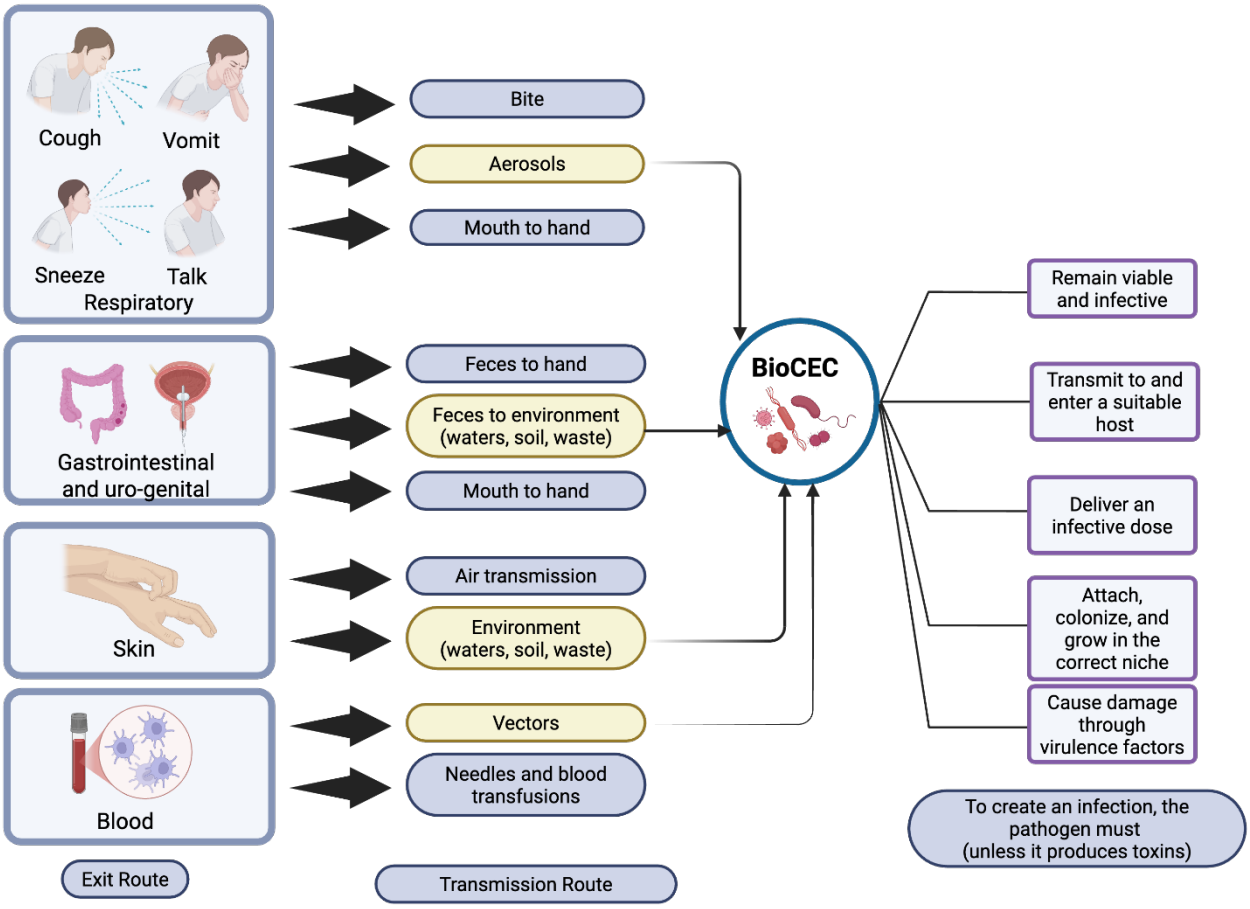

Figure 4-4. Exit and transmission routes for pathogens to transmit to a new host.

Pathogens usually rely on airborne, foodborne, waterborne, and/or vector-borne transmission pathways. Pathogens are also able to move directly from person to person or animal to person (i.e., zoonotic transmission) or indirectly from a contaminated, inanimate surface to a person (i.e., fomite transmission). Some pathogens can also be vertically transmitted to an embryo or fetus, which is not within the scope of this guidance (Mara and Horan 2003). Pathogen success requires survival in the environment at large enough numbers to cause illness; this is critical to pathogen success. **Figure 4-4** highlights some of the pathways and requirements for infection to occur. The figure is followed by summaries of some variables to consider when evaluating airborne and waterborne pathogens and descriptions of the factors that influence pathogen persistence.

4.3.4.1 Airborne Transmission

The COVID-19 pandemic has sparked a lot of interest in respiratory infectious disease. A broad range of microorganisms (e.g., viruses, bacteria, fungal spores) from an infectious or environmental source may disperse over long distances by air currents and ultimately be inhaled or ingested. Two main factors drive airborne pathogen transmission: **particle size** (i.e., the diameter of the particle) and the extent of desiccation (Cole and Cook 1998). The particle size controls whether the particle becomes and remains airborne and infectious. Large particles fall out of the air quickly, while small particles remain airborne for a longer time. The WHO uses a particle diameter of 5 micrometers (µm) to delineate between airborne (≤5 µm) and droplet (>5 µm) transmission. Studies indicate larger particles (6 to >10 µm) deposit in the upper airway, while smaller particles penetrate deeper into the respiratory tract, or alveolar region (Darquenne 2012; Fernstrom and Goldblatt 2013). These size-based distributions are not always clear cut. Droplets settle out of air onto a surface at a velocity dictated by their mass, but in some indoor environments, air currents alter the particle's velocity thus keeping the particle airborne.

Another critical variable is the rate at which particles desiccate. Rapid desiccation is a concern since the smaller and lighter the infectious particle, the longer it will remain airborne and the farther it will travel. Infectious agents can be expelled from the respiratory tract in a mixture of mucus and secretions, creating large, heavy particles, but rapid desiccation can lengthen the time they remain airborne as dried residuals (known as droplet nuclei, typically in the 0.5–12 µm range). Also, very large aerosol particles may initially fall out of the air only to become airborne again once they have desiccated, thus emphasizing the importance of disinfection and hygienic practices to reduce transmission (Fears et al. 2020; Fernstrom and Goldblatt 2013).

Factors such as temperature, humidity (both relative and absolute), sunlight (ultraviolet light) exposure, and even atmospheric pollutants can all act to inactivate airborne pathogens (Tang 2009). It is important to note that temperature and humidity influence viral, bacterial, and fungal particles differently. Generally, as temperature rises, virus survival decreases. Bacteria are more resistant to temperature than viruses. Temperatures above 24°C (75.2°F) typically are required to reduce airborne bacterial survival, but this is highly species dependent (Karra and Katsivela 2007). Relative humidity is recognized as a factor in the viability of airborne and droplet viral transmissions; however, the exact relationship is currently not well understood (Fernstrom and Goldblatt 2013). Fungi and their spores appear to be more resilient than viruses and bacteria, as they are often able to withstand greater stresses due to dehydration and rehydration, as well as UV radiation (Cole and Cook 1998).

4.3.4.2 Waterborne Transmission

The persistence of pathogens in water is influenced by many factors including temperature, exposure to sunlight (UV), predation, and certain chemical conditions (e.g., salinity, dissolved organic carbon), as well as settling or interactions with sediment (Brookes et al. 2004). A review by Dean and Mitchell (2022) cautioned that using different detection methods (i.e., culture versus molecular) may provide different results in terms of pathogen persistence. They also found that water type did not consistently affect decay, but fecal indicator bacteria decay faster in water than sediment. In addition, turbidity and

temperature were found to be significantly and positively associated with indicator bacteria decay; however, sunlight and pH did not have statistically significant correlations. Overall, the effect of sunlight was more pronounced during the initial stages of decay, and over the course of time biotic interactions (i.e., predation) had a greater influence on decay than sunlight (Dean and Mitchell 2022). Generally, persistence of waterborne pathogens tends to be complicated.

4.4 Approaches to BioCEC Prioritization Strategies

This subsection describes different existing prioritization strategies that are widely applied to identify key variables and characterize risk from BioCEC. These strategies may inform efforts to proactively identify and develop strategies to address BioCEC. The prioritization approaches from the below examples from Health Canada, WHO, and USEPA were used in the following **Tools for Prioritization** to develop a guide for selecting a suitable prioritization method.

4.4.1 Health Canada

A QMRA is conducted to assess the potential microbiological risks associated with an infrastructure system. QMRA is a scientific approach used to estimate the risk of illness from exposure to a pathogen. Health Canada developed a QMRA model for drinking water systems and published guidance to describe their approach (Health Canada 2018). Although QMRA is useful as a quantitative method to assess risk and is commonly applied as a computer-based mathematical model, the QMRA perspective can be helpful in a range of applications from qualitative to quantitative. The Health Canada guidance includes general information about QMRAs, specific guidance for the Health Canada Excel-based QMRA, and a QMRA case study for a municipal water treatment plant. The following paragraphs provide more detail from the Health Canada guidance.

QMRA models assess potential risks associated with bacterial, protozoan, and viral pathogens. Assessment results are used by regulatory agencies and drinking water authorities to quantify health risks from microorganisms in water sources and develop drinking water guidelines for enteric viruses and protozoa. The approach uses the traditional hazard identification framework of exposure assessment—dose–response assessment—risk characterization. Health Canada applies QMRA across the entire drinking water system from source water to consumer.

The range from qualitative to quantitative application would start with items such as yes/no checklists (qualitative), incorporate risk matrices (semi-quantitative), and include screening assessments and probabilistic assessments with uncertainty analysis (quantitative). On the qualitative end of the spectrum, simple checklists can yield high, medium, and low risk prioritizations by tallying answers to yes/no questions. The Health Canada QMRA is especially useful at the screening assessment level for a water treatment system but is not appropriate for more advanced studies such as probabilistic assessments.

Health Canada notes that pathogen data should be considered carefully due to the small size of datasets, potential for low pathogen density, and the episodic nature of pathogen loading. For these reasons it can be difficult to characterize variability in a system. These characteristics might set BioCEC apart from other contaminants of emerging concern such as chemical contaminants. As a result, many facilities tend to rely heavily on expert judgment and literature values.

The **Health Canada Excel model** uses inputs such as source water pathogen concentrations, treatment system type, and ingestion/dose–response values from literature. Model results are then used to estimate the annual risk of infection and illness. The case study included in the Health Canada guidance describes a scenario wherein a municipal water treatment plant draws water from a large river that is situated in a rural, agricultural district. Decision-makers at the plant are interested in how variations in raw water quality as well as disinfection methods will affect their processes and the associated risk of

infection. The case study results indicate that physical removal and disinfection processes are necessary for control of certain pathogens, and that chlorine and ozone appear to offer negligible benefits. These results offer an example of the usefulness of the Health Canada QMRA approach and can be used as a starting point for further analysis.

4.4.2 World Health Organization Guideline

The intended audience of the WHO report, "Quantitative microbial risk assessment: application for water safety management," (WHO 2016) was scientists, regulators, and water supply and sanitation system engineers and managers. The WHO report was meant to support raising awareness around how QMRA works, when it provides value for water safety management, and how it should be applied in water systems specifically. It provides detailed examples of how to use QMRA and best practices associated with the assessment based on the concept that risk assessment plays a central role in the implementation of a preventive, risk-based approach to water quality management from source to exposure for the management of microbial hazards. The document acknowledges the varying levels of expertise, data, and resources required to implement a risk-based approach. As a result, it provides several options for assessment, ranging from risk scoring in sanitary inspections and risk matrices to QMRA (Figure 4-5). These options need to be explored when trying to manage risk from an environmental pathogen to ensure the most suitable approach is selected.

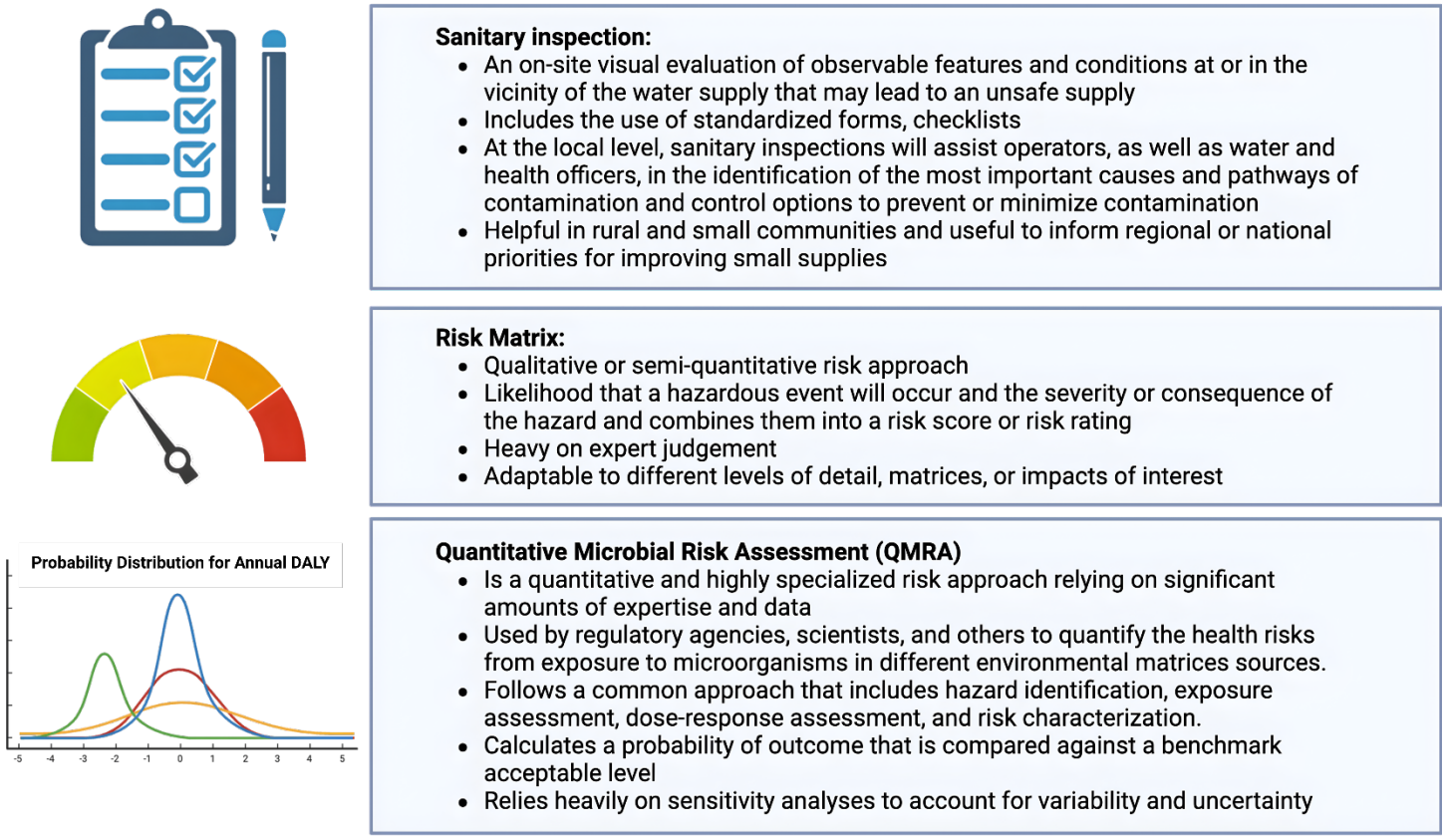

Figure 4-5. Risk assessment options available for risk mitigation.

4.4.2.1 The QMRA Framework

While exposure to infectious agents is not a new risk, all three components of the epidemiological triangle (i.e., the pathogen, host, and environment) are constantly changing, potentially impacting the risk

of infection and associated risk mitigation strategies. Infectious risk statistics and indicators are for the most part measured as rates (i.e., represent an X number of individuals that have the disease per 1,000 individuals over some unit of time). This value depends on the efficacy and reliability of the disease surveillance program and has greatly underestimated the number of infections in a community.

Recognizing the deficiencies of epidemiology to characterize risk, scientists started using mathematical approaches to evaluate risk in the 1980s. These methods were further developed over the last four decades and now provide a broad range of tools to practitioners who need to perform risk assessments. QMRAs are fairly versatile and can be used to assess the potential for human risk from exposure to a known pathogen; determine critical control points in the different systems; compare specific risk mitigation, disinfection, or treatment process options to reduce the levels of various pathogens; identify and prioritize research needs; and assist in epidemiological investigations. The WHO document provides several case studies that touch on different QMRA objectives that may be of interest to the reader (see **Table 4-2**, adapted from WHO 2016).

Table 4-2. Summary of case studies in the World Health Organization quantitative microbial risk assessment framework.

Risk Management Objective	Case Study		Reference
Evaluation of hazards	#1	Pathogen risk to swimmers at non-sewage-impacted recreational beaches in the US	(Schoen and Ashbolt 2010)
Evaluate alternative options	#2	Water reclamation redesign for reducing *Cryptosporidium* risks at a recreational spray park in the US	(Weir et al. 2011)
Determine priorities	#3	Evaluating *Cryptosporidium* risk at a large number of drinking water systems in France	(Medema et al. 2009)
Cost-benefit	#4	USEPA Long Term Surface Water Treatment Rule — health benefit of new drinking water regulation in the US	(USEPA OW 2005)
Setting health-based performance targets	#5	Guidelines for water recycling — setting health-based performance targets and safe use of wastewater in Australia	(NWQMS 2006)
	#6	WHO health-based criteria for evaluating household water treatment technologies	(WHO 2011)

Source: Adapted from (WHO 2016).

The WHO QMRA framework defines four specific components to all QMRAs (**Figure 4-6**): problem formulation, exposure assessment, health effects assessment, and risk characterization. This framework is similar to the traditional approach defined by others (Haas et al. 2014), which includes hazard identification, dose–response assessment, exposure assessment, and risk characterization. Some references specifically include risk management and communication steps at the end of the QMRA process, which can be highly desirable and useful.

The problem-formulation phase defines the scope of the assessment and the overall context (i.e., the reference pathogens, exposure pathways, hazardous events, and health outcomes of interest). This would also be the stage that focuses on hazard identification. Hazard identification includes the identification of both the agent of infection and the disease outcomes. Traditionally, these outcomes range from asymptomatic infections to death, and they are highly related to pathogen virulence properties. Endemic and epidemic disease outbreak investigations, case studies, hospitalization data, and other epidemiological data sources are needed to complete this assessment. It is important to note that transmission patterns and probabilities are pathogen specific.

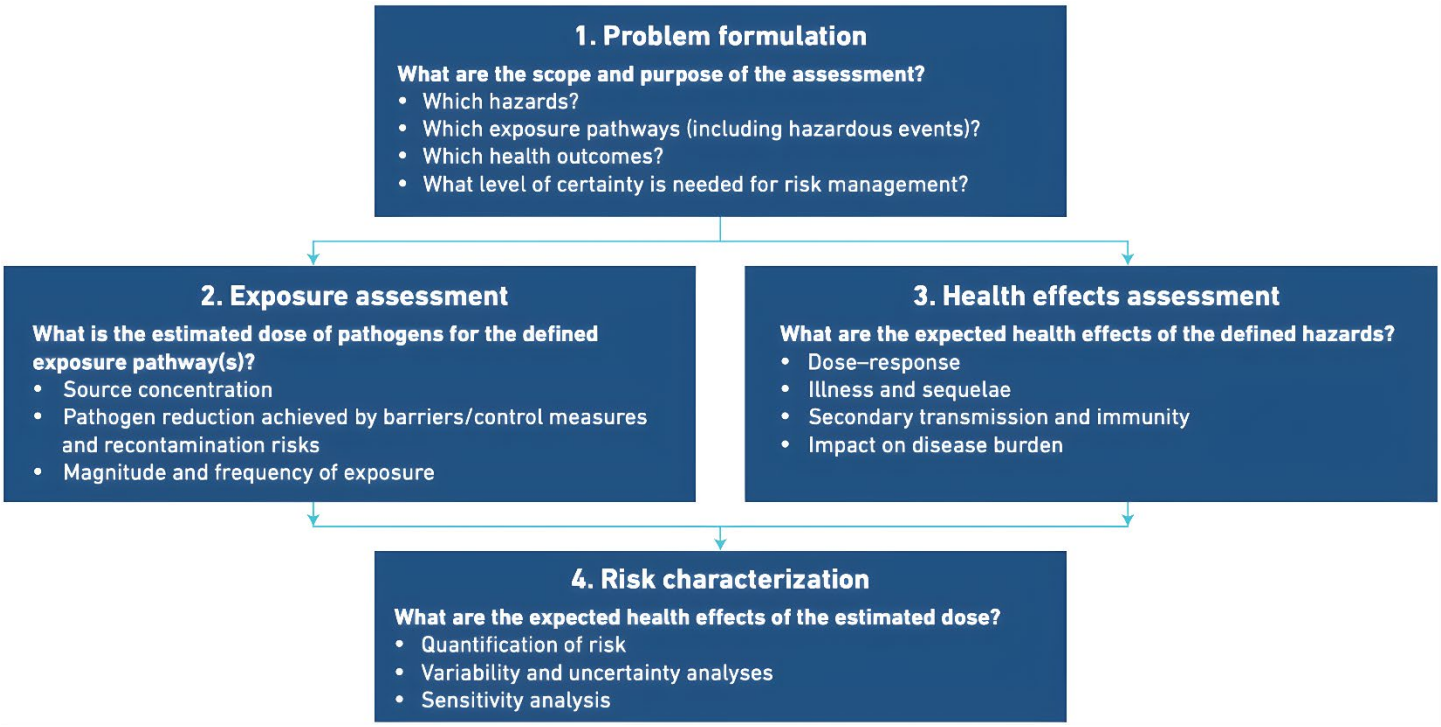

Figure 4-6. The World Health Organization quantitative microbial risk assessment framework.

Source: (WHO 2016).

The objective of the exposure assessment step is to determine the frequency and magnitude of exposure to pathogens via the pathways and hazardous events defined during problem formulation. Critical quantitative information includes pathogen concentrations in environmental matrices or the fate, persistence, or decay of pathogens in barriers (e.g., treatment processes) during normal and incident situations. But other data related to exposure of humans (e.g., the size of the exposed population, vulnerability of the exposed population, frequency of exposure) are also needed.

The health effects assessment involves identifying the health impact data for the identified hazards and the specific study population. This includes the type of health effects (including secondary and/or chronic health effects that occur following initial infection), the severity and duration of illness that may occur after exposure to the pathogen, and available information on the relationship between ingested dose and the probability that health effects (infection or illness) occur (dose–response relationship). Also, the fraction and vulnerability of the population exposed may need to be considered in addition to secondary transmission and immunity. Dose–response is also a component of the health effects assessment and the mathematical relationship between the dose administered and the probability of infection or disease in the study population. The microorganisms are routinely counted in "numbers" used to count them in laboratory studies (e.g., plaque forming units, colony-forming units, (oo)cyst counts in microscopy). In dose–response experiments, traditional exposure pathways are used to measure disease and infection as endpoints. These experiments are not without limitations as culture-based assessments underestimate pathogen concentrations and molecular methods overestimate concentrations. Also, most studies use healthy individuals and less virulent pathogen strains, and few studies assess multiple combined exposure pathways.

Finally, risk characterization is the synthesis of all the information from the exposure and health effects assessments to produce a probability of occurrence and severity of adverse health effects in an exposed population. Risk characterization encompasses four distributions: (1) the spectrum of health outcomes;

(2) the confidence limits around the dose–response model; (3) the distribution, occurrence, and viability of pathogens; and (4) the exposure distribution. Sensitivity analyses are recommended due to the uncertainty in the QMRA to determine the variability and uncertainty in the information at each individual step of the risk assessment and how it affects the overall risk estimate. The risk characterization is either deterministic (meaning that single values such as means are used to describe the variables used in the QMRA model) or probabilistic (meaning that statistical distributions are used to describe variables used in the QMRA model). In a deterministic QMRA, estimates of each of the QMRA model variables in the exposure and effects assessment are selected and combined to compute the resulting health risk. In a probabilistic QMRA, statistical distributions are used to describe the model variables, which reflects the stochastic (variable/uncertain) nature of most of the model variables more appropriately. The type of distribution selected considers a combination of knowledge of (pathogens in) water systems and of statistics. The health risk is computed by combining the statistical distributions using Monte Carlo methods. A wide range of software tools is available to support these calculations. Dedicated QMRA models and software tools are increasingly available to aid this risk characterization step.

Although calculated risks can be compared against a health target, users of QMRA should keep in mind that QMRA does not calculate actual disease outcomes but provides a probability that disease may occur in a specific scenario under specific circumstances. The time scale in which the risk is expressed may differ, from single-exposure events to all exposures in a year. The risk may be quantified with different endpoints, including the probability of infection, probability of illness, expected number of illness cases, and measures for burden of disease, such as disability-adjusted life years (DALYs). The need to conduct a deterministic, screening-level QMRA or a probabilistic, in-depth QMRA is primarily determined by what is needed to determine the best risk mitigation options.

The main strength of QMRA is that it is evidence-based, replicable, objective, and transparent, which allows for the discernment between risks when compared with other risk assessment approaches. Although other risk assessment approaches can also provide justification for investments in data collection and analysis improvements, QMRA can provide a more precise justification, which may be particularly useful when significant investments are required. QMRA provides a holistic system understanding; it considers all the components in the system and provides valuable information on the effects of each component on the risk of human disease associated with exposure to the pathogens. The results provide a scientific basis for evaluation of risk management priorities or control strategies. Further, QMRA enables the development of performance and specific technology targets, to determine whether or not microbial health outcome targets can be met.

4.4.2.2 What Does Disability-Adjusted Life Years (DALY) Mean?

Within the risk assessment literature, a number of mortality/morbidity metrics are used internationally that address human burden of diseases. These include years of life lost, quality-adjusted life years, disability-adjusted life expectancy, healthy life years, and DALYs (Kobayashi et al. 2015). The DALY is the metric used in WHO guidelines for the overall community health burden. The DALY is a summary measure of population health that incorporates the different severities and durations associated with different illnesses. The DALY has been applied as a metric within the WHO guidelines to provide a different relative weight to pathogens based on severity of disease outcomes. DALYs are particularly comprehensive because, in addition to the number of deaths caused, they account for years of life lost due to premature death, severity and duration of morbidity, and the number of individuals affected (Murray and Acharya 1997). It is important to include the variability (natural dispersion in a system, such as pathogen concentrations in a river) and uncertainty (lack of understanding and/or inability to measure) in all steps of the risk characterization.

One DALY represents the loss of one healthy life year. For each identified health outcome in a QMRA, DALYs are calculated as the sum of the years lost due to premature mortality and the years of productive life lost due to disability for incident cases of ill-health conditions (i.e., DALY = years of life lost + years

living with a disability). Although the term disability has many meanings in different contexts, here disability refers to any short-term or long-term loss of health. To be able to calculate a DALY for each hazard or health risk, the assessor needs to identify disease outcomes to be considered (construct an outcome tree), determine the number of cases for each outcome in the population (estimate the probability associated with each outcome), identify the duration of response (years of life lost), and estimate the severity of response (Schoen et al. 2023). The WHO uses a threshold of 10^{-6} DALYs per person per year as a benchmark to set water reuse and drinking water treatment requirements. Other organizations have used a probability of infection benchmark of 10^{-4} per person per year in the US for drinking water treatment requirements and for potable reuse in the State of California (Schoen et al. 2023).

DALYs are subject to broad debate related to methods and efforts to quantify outcomes in economic equivalents of health interventions. For example, the capacity of individuals with some chronic disorders to adapt to their circumstances could lead to the underestimation of the health loss associated with a particular state (Salomon et al. 2012). In addition, the impact of disability within particular social and cultural environments can differ significantly, thus raising questions about the possibility of significant cross-cultural variability in disability weights (Salomon et al. 2012).

4.4.2.3 Best Practices in the WHO Document

The WHO (2016) document outlines a few best practices and recommendations. For example, when relying on literature alone without site-specific data, it is often necessary to be conservative, and therefore high numbers need to be selected. In addition, relying on a sophisticated statistical analysis without holistically thinking about data inputs and assumptions would be an inadequate evaluation of system risks. Quantitatively accounting for these types of uncertainty is a challenge; however, a transparent approach to scenario analysis with point estimations provides a useful tool. Finally, in each case, these scenario calculations should be run in parallel with the “best” estimate calculations — they should not replace the best estimates. Using the upper limit of uncertainty at every stage of the model would provide a risk estimate that is unmanageably conservative and not truly representative of the population and most likely would not be very helpful for risk management. Alternatively, comparing uncertainty scenario results with the best estimates can provide useful inputs regarding model sensitivity and robustness.

4.4.3 US Environmental Protection Agency Contaminant Candidate List 5 for Drinking Water Supply

4.4.3.1 Overview

The USEPA is required by the 1996 Safe Drinking Water Act amendments (section 1412(b)(1)) to publish a list of drinking water contaminants that may cause adverse health effects in humans that are known or anticipated to occur in public water systems (USEPA OW 2022). This CCL is published approximately every five years and identifies priority contaminants for regulatory decision-making and for prioritizing research and data collection efforts. The USEPA published the first CCL for microbial contaminants, CCL 1, in 1998, and its most recent iteration, **CCL 5**, was published in November 2022. The CCL identifies a list of contaminants to be considered for data collection efforts and research for the Unregulated Contaminant Monitoring Rule. Contaminants are eventually considered for regulatory determination and rulemaking under the Safe Drinking Water Act only after additional data and information are collected.

The National Research Council assisted the USEPA in the development of a more robust framework for identifying and prioritizing drinking water contaminants from CCL 3 onwards. The CCL framework now consists of the following three steps (Figure 4-7):

- Step 1: Build a broad "universe" inclusive of all microbes that may cause human disease.
- Step 2: Screen the universe using specific exclusion criteria to generate a preliminary CCL (PCCL).
- Step 3: Score and rank the PCCL contaminants based primarily on their occurrence in drinking water and their health risk to finally yield the CCL.

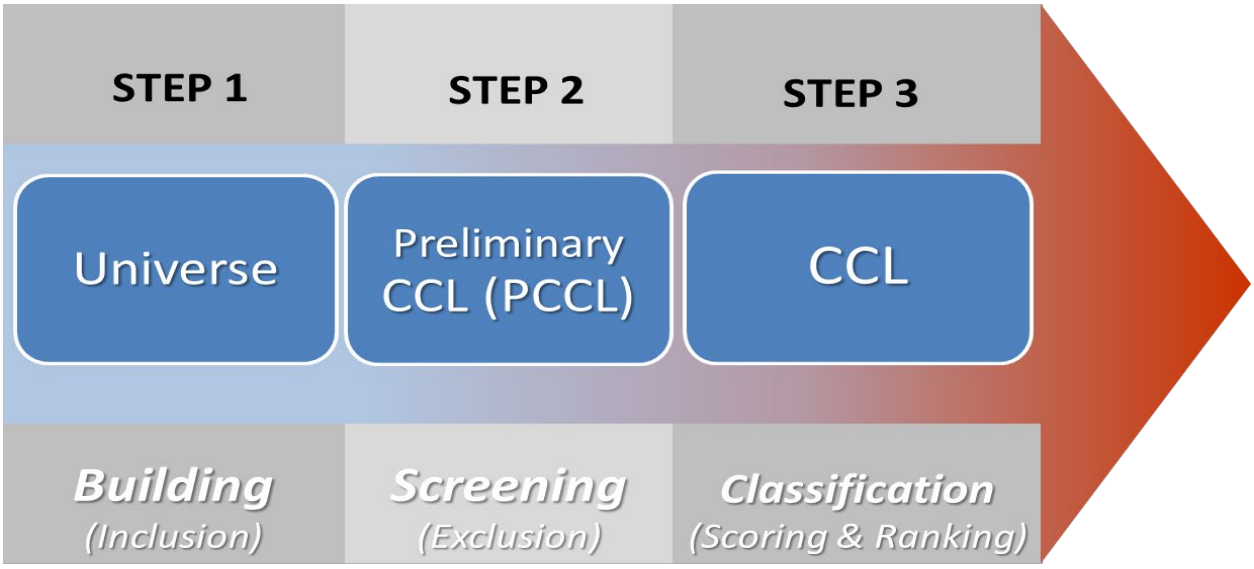

Figure 4-7. The Contaminant Candidate List (CCL) framework since CCL 3.

Step 1 uses a combination of literature review, input from subject matter experts, and public nominations to yield a universe of microbial contaminants capable of causing human disease. Step 2 uses 12 exclusion criteria against the contaminants in the universe to generate the PCCL. Step 3 scores the contaminants in the PCCL based on occurrence and health risk and is then ranked to give the final CCL. These steps are described in more detail in the subsections that follow.

Step 1: Building the Universe

The USEPA, upon the recommendation of the National Drinking Water Advisory Council (NDWAC 2004), began to specifically use Taylor et al. (2001), a comprehensive literature review identifying species of infectious organisms known to be pathogenic to humans, and more recent literature reviews as the starting point for building the microbial universe for CCL 3. This resulted in a total of 1,425 microbes in the universe for CCL 3: 1,415 from Taylor et al. (2001), and 10 from additional literature reviews. These 1,425 pathogens have been carried forward in the subsequent CCL 4 and CCL 5, with the added step of seeking feedback from subject matter experts. Starting with CCL 5, a new phase of seeking public nominations (Figure 4-8) was introduced to the universe building stage. The public nominations committee asked for the name of the pathogen and for data evidence, such as the pathogen's likely occurrence in public water systems and its health effects, that would make it potentially require regulation. The public nomination phase yielded 16 unique microbial contaminants for consideration for CCL 5. CCL 5 generated a universe consisting of 1,435 microbes after minor changes to nomenclature and consolidation of some members.

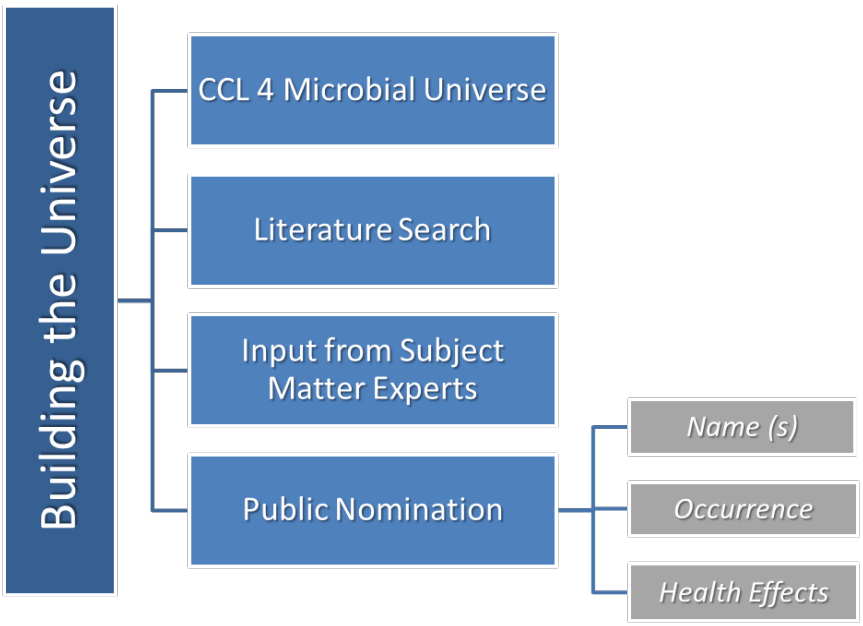

Figure 4-8. Step 1 of the Contaminant Candidate List 5 framework: building the universe.

Source: Adapted from (USEPA OW 2022).

Step 2: Screening

For the screening step, 12 exclusion criteria were developed to screen the microbial universe developed in Step 1. The exclusion criteria focused on plausibility of pathogen presence, survival, and transport through drinking water to disease manifestations from drinking water exposure (USEPA OW 2022). The specific exclusion criteria were as follows:

1. Anaerobes (microorganisms that cannot survive in oxygenated environments)
2. Fastidious or obligate intracellular pathogens (environmental survival in water implausible)
3. Pathogens exclusively transmitted by direct or indirect contact with blood or body fluids (including sexually transmitted diseases)
4. Pathogens transmitted by vectors
5. Microflora common to the gastrointestinal tract, skin, and mucous membranes
6. Pathogens transmitted solely by respiratory secretions
7. Pathogens whose life cycle is incompatible with drinking water transmission
8. Pathogens where drinking water–related transmission is not implicated
9. Natural habitat is in the environment without epidemiological evidence of drinking water–related disease and without evidence of drinking water–related nosocomial (i.e., hospital-based) infection
10. Pathogens not endemic to North America

11. A genus and species or serotype may be chosen to represent a group of closely related organisms

12. Current taxonomy does not support the classification listed by Taylor et al. (2001)

The screening step eliminated 1,400 of the 1,435 pathogens, leaving only 35 pathogens on the PCCL (Table 4-3).

Table 4-3. Pathogens screened from the universe using exclusion criteria.

Pathogen Class	Total (Universe)	Pathogens Excluded	PCCL
Bacteria	545	527	18
Viruses	225	218	7
Protozoa	66	59	7
Helminths	286	286	0
Fungi	313	310	3
Total	1,435	1,400	35

Note: PCCL is preliminary contaminant candidate list.

Source: Adapted from (USEPA OW 2022).

Step 3: Classification

The classification step takes the PCCL generated in the screening step and puts each pathogen on that list through a scoring system focusing on two categories: (1) its occurrence in public water systems and (2) its health risk for causing adverse health effects in humans. The occurrence scoring relies on a combination of waterborne disease outbreak (WBDO) data from the CDC's Morbidity and Mortality Weekly Reports (Benedict 2017) or pathogen occurrence data in drinking water and source water. The higher of the two scores (either WBDO or occurrence in drinking/source water) is selected for the occurrence criteria. The WBDO scoring follows a five-level hierarchy (i.e., scores ranging from 5 to 1) using the following classifications:

5: Has caused two or more WBDOs in the US as reported by the CDC between 2009 and 2017.

4: Has caused at least one WBDO in the US as reported by the CDC between 2009 and 2017.

3: Has caused documented WBDOs any time in the US.

2: Has caused documented WBDOs in countries other than the US.

1: Has never caused WBDOs in any country but has been epidemiologically associated with water-related disease.

Pathogen occurrence in drinking/source water scoring follows a three-level hierarchy:

3: Detected in drinking water in the US.

2: Detected in source water in the US.

1: Not detected in the US.

From the range of hierarchies listed above, it is obvious that WBDO data can result in a higher score for the first term in Equation 4-1 than simply the occurrence data for the pathogen in drinking/source water.

$$\text{Total Score} = \text{Highest Score, WBDO or Occurrence} + \left[\left(\text{General Population Score} + \text{Highest Sensitive Population Score}\right) \times \frac{5}{14}\right]$$

Equation 4-1.

The health risk scoring follows a 7-level scoring hierarchy for the general population, as well as four sensitive subpopulations: children/infants, the elderly, pregnant women, and people with chronic disease. The highest score of 7 is given when there is significant mortality involved (>1/1,000 cases) while the lowest score of 1 is reserved for mild symptoms with minimal or no impact on daily activities. The summative score for the health risk for the two populations (i.e., second term of Equation 4-1) is then normalized by a correction factor representing the five types of populations divided by 2× the score range for the two populations.

All 35 pathogens on the PCCL were scored using the criteria described above, and then a total score was calculated for each using Equation 4-1. After sorting the 35 pathogens from highest to lowest total score, the top 12 pathogens were selected as the final pathogens for CCL 5 (Table 4-4).

Table 4-4. Final Contaminant Candidate List 5 consisting of the 12 highest-ranked pathogens.

Pathogen	Ranking	WBDO	Occurrence	Health	Total Score
Naegleria fowleri	1	5	3	5.0	10.0
Legionella pneumophila	2	5	3	3.6	8.6
Escherichia coli (O157)	3	5	3	3.2	8.2
Pseudomonas aeruginosa	4	5	3	3.2	8.2
Helicobacter pylori	5	1	3	5.0	8.0
Campylobacter jejuni	6	5	3	2.5	7.5
Mycobacterium abscessus	7	4	3	3.2	7.2
Shigella sonnei	8	4	3	3.2	7.2
Caliciviruses	9	5	3	2.1	7.1
Mycobacterium avium	10	4	3	2.9	6.9
Adenovirus	11	2	3	3.6	6.6
Enterovirus	12	2	3	3.6	6.6

Note: WBDO is waterborne disease outbreak.

Source: Adapted from (USEPA OW 2022).

4.4.3.2 Limitations

A number of limitations exist in the CCL 5 prioritization framework. Specifically, exclusion criteria 1, 9, and 10 have been previously challenged by the CCL 4 Science Advisory Board. For example, exclusion criterion 1 was challenged because of the presence of spore-forming anaerobes that survive oxygenated

environments. For exclusion criterion 9, nosocomial infections were included in the criterion from CCL 4 to CCL 5, and concern for the presence of pathogens in distribution systems was recognized owing to factors such as biofilms. Criterion 10 remained unchanged after further review by the USEPA. Lastly, the WBDO disease outbreak data from the CDC is largely believed to be underreported for multiple reasons, including the variability in surveillance capabilities across states.

4.5 Tools for Prioritization

Several tools can be used to understand the risk associated with BioCEC including surveys, a risk matrix, or QMRA (WHO 2016). The selection of a tool to use depends on the expertise of the individuals conducting the risk assessment, their experience with advanced statistical assessment, and the availability of data. For some BioCEC, the limited availability of data necessitates the use of a qualitative or semi-quantitative approach; however, the use of qualitative and semi-quantitative approaches is still powerful. A guide to selecting the type of approach to apply is shown below in a flowchart (Figure 4-9).

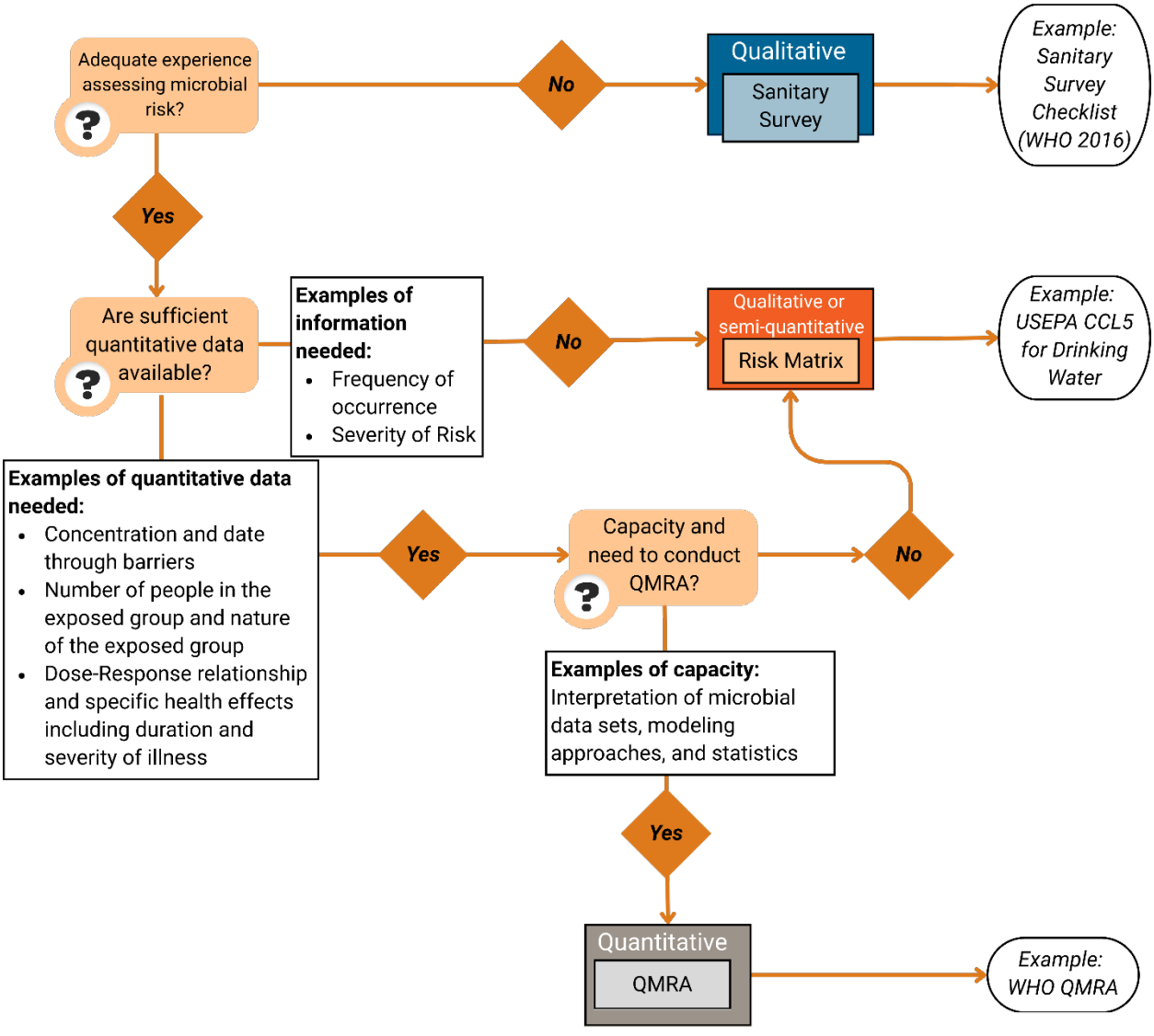

Figure 4-9. Approaches applied to assess the risk of biological contaminants of emerging concern.

Surveys for evaluating BioCEC, for example, sanitary surveys, may be generated in-house or by a national or international organization. Although sanitary surveys are traditionally used in water supply systems in the US, a sanitary survey can also be used as a more holistic method to investigate the sources of fecal contamination to a waterbody. Sanitary surveys can be used for drinking water, shellfish, and watershed protection programs, as well as beaches and other recreational waters. Surveys or checklists can be developed to qualitatively compare risks associated with different BioCEC at a specific site. It allows operators or inspectors to provide a binary answer to questions about the presence of specific BioCEC (yes or no) and to broadly classify the risk associated with each (from very high risk to low risk). Surveys can also be used in conjunction with environmental parameters (e.g., water quality data) to provide additional means of prioritization based on data. The strength of a survey or checklist is that it is straightforward and requires the fewest resources to complete, which also means that it may be feasible to update more frequently. A limitation of the survey approach is that it may not capture all potential hazards.

The next step up in complexity and knowledge required is a risk matrix, which can be either qualitative or semi-quantitative (Fewtrell and Bartram 2001; WHO and International Water Association 2009). In the qualitative approach, experts can classify the risk from a given BioCEC based on its severity or consequence and the likelihood or frequency. In the semi-quantitative approach, different BioCEC are given scores that can then be totaled and ranked. This semi-quantitative approach was used by the USEPA to rank the list of BioCEC to generate the Microbial CCL5 (USEPA OW 2022).

The advantage of a risk matrix is that it can capture different types of risks and tends to cover a wider range of events relative to a survey or checklist. It can be challenging to be consistent in the application of risk scores for different types of hazards and to determine the likelihood of different events.

QMRA is a systematic approach to decision-making and prioritization of risks. Examples of the types of data required for completing a QMRA include understanding the BioCEC occurrence, variability, and fate through different barriers. The analysis also includes the number of people exposed to the pathogen, the various routes of exposure, and the type of people in the exposed group, such as the elderly or children. In addition, the dose–response relationship and specific health effects, including duration and severity of the illness, are needed to complete a QMRA. Relative to a survey or risk matrix, the QMRA provides the most systematic and rigorous approach to the comparison of different risks; however, the QMRA requires significant data, statistical knowledge, and expertise.

4.6 Limitations and Knowledge Gap

The Interstate Technology and Regulatory Council's BioCEC team conducted a nationwide survey with the goal of understanding current practices in different states. One of the questions asked during this survey was how states and agencies approach the prioritizing and monitoring of BioCEC. The responses indicated that there are significant variations between the responders with the majority relying on federal guidance and requirements. Most states and agencies do not have a prioritized list of BioCEC, while some states, such as Alabama and New Hampshire, have partial prioritization schemes (that focus on specific contaminants), such as harmful algal blooms and *Legionella*. When prioritization occurs, it is influenced by factors such as federal guidance, resource availability, risk to public health, and federal requirements. In terms of monitoring, a majority of the states are not monitoring for unregulated BioCEC, while some states such as Vermont are monitoring for specific unregulated BioCEC related to site-specific events, such as cyanobacterial blooms. In Utah, spot monitoring is conducted for unregulated BioCEC when there is a reason for concern, such as reported outbreaks. Common triggers for starting monitoring programs were identified as federal requirements, known or suspected releases, public health concerns, and availability of funding. Although monitoring programs and collecting data are key to evaluating BioCEC, the primary limitations for widespread monitoring programs are available resources and funding.

Different prioritization schemes were presented in this guidance, including WHO's guideline and USEPA's CCL5 list. Although these resources provide generalized best practices and recommendations to use qualitative and quantitative data or information to prioritize BioCEC, evaluation of specific risk may be highly site and scenario specific. This requires significant expertise and resources when developing a strategy or approach to evaluate risk. In addition, criteria developed to define a prioritization approach, such as exclusion basis, could be debatable, and it may be difficult to have full agreement among subject experts, as presented in **US Environmental Protection Agency Contaminant Candidate List 5 for Drinking Water Supply** on CCL5. Some of the tools used to evaluate risk, such as QMRA, require significant data and information, such as established dose–response models and occurrence data. Particularly with emerging BioCEC, this information may not be readily available in the literature and may require research and significant resources to conduct monitoring campaigns and establish dose–response models. In those cases, to make timely decisions to protect public health, relevant surrogate pathogen data can be used to estimate persistence, dose–response, or other variables until BioCEC data become available. For example, the Water Environment Federation COVID-19 guidance for water and wastewater systems relied on surrogate viruses to estimate risk (Water Environment Federation 2020). In the use of QMRA to evaluate water reuse, the USEPA identified data on pathogen densities in source water, viral load estimation methods that inform viability and infectivity, lack of dose–response data for enteric viruses, and understanding health burden (Jahne et al. 2025) associated with reference pathogens as some of the critical knowledge gaps. Overall, the number of key variables that correspond to a high-risk BioCEC are vast, complex, and highly interlinked, which makes prioritization of BioCEC a highly involved and multi-step process as presented in **Approaches to BioCEC Prioritization Strategies**.

5 ANALYTICAL METHODS

This section describes methods that could be used for detecting and quantifying **biological contaminants of emerging concern (BioCEC)** in various environmental matrices (e.g., air, water, and soil) and **vectors** (Figure 5-1, see also Conceptual Exposure Model subsection 1.3 Defining the Environment). Reliable detection of a **pathogen** in environmental matrices and vectors requires a high degree of confidence in its identity and quantitation. Specific matrix subtypes are listed in Table 5-1. In addition to environmental matrices and their subtypes, Table 5-1 includes selected references and resources detailing sampling methods for each matrix. Different environmental matrices require specific sampling techniques to ensure representativeness and data quality and to avoid bias or error. Environmental sampling typically involves the development of a sampling plan (e.g., how, when, and where samples will be collected and how many), physical collection of samples, transport and storage prior to analysis, and then analysis.

Designing and choosing an appropriate sampling method is complex and depends on many factors including study objectives, environmental matrix type, BioCEC characteristics, analytical method used, and available resources, to name a few. Thus, specific and comprehensive sampling methods and design plans for BioCEC are challenging to prescribe. Numerous resources cited here are publicly available and are continuously being updated and improved in recognition of the important step of sampling in BioCEC identification (see USEPA ORD 2024, Table 5-1, USEPA OW 2025; Zhang 2007). Designing and selecting an appropriate sampling method is critical prior to applying any analytical method used for BioCEC detection. This will most likely require engagement among decision-makers, public health officials, laboratory personnel, subject matter experts, and other relevant stakeholders to ensure analytical recovery, accuracy, and reliability.

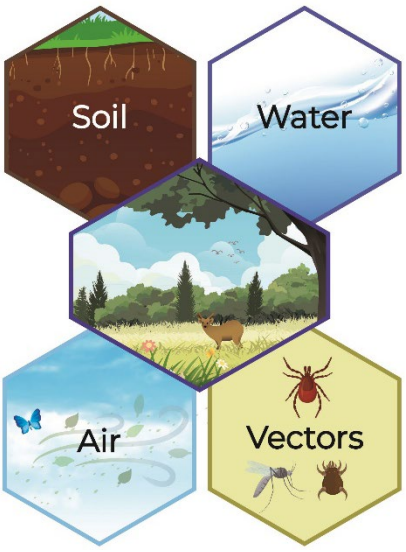

Figure 5-1. Graphical depiction of environmental matrices.

Table 5-1. Sampling guidance and resources by environmental matrices type and subtype.

Environmental Matrix Type	Matrix Subtypes	Relevant References and Resources
Air/aerosol	Various sizes of airborne particles or aerosols	Outdoor bioaerosol sampling onto agar plates (Kobziar et al. 2018) Indoor aerosol sampling (Kumar et al. 2021) Aerosol sampling for disease surveillance (Santarpia et al. 2023)
Water	Drinking water Freshwater Surface water Brackish water Saltwater Stormwater Wastewater	Drinking water: Standard Methods for the Examination of Water and Wastewater (APHA et al. 2023) Freshwater and surface water monitoring and sampling guidance (BC Ministry of Environment, Lands and Parks. Water Management Branch., n.d.; Queensland Government 2018); US Environmental Protection Agency (USEPA) national aquatic resources for surface water sampling methods (USEPA OW 2025) Brackish water and saltwater: guidance on water quality criteria, monitoring, and sampling (USEPA OW 2014; CAEPA 2019) Stormwater: Saifur and Gardner (2021) discuss the significance of stormwater microbial contamination and detection of antibiotic-

Environmental Matrix Type	Matrix Subtypes	Relevant References and Resources
		resistant genes in recreational and receiving water bodies Wastewater: (Alygizakis et al. 2020); New York state COVID Resources doc
Soil/Sediments	Sand Silt Clay	Soil sampling (Kobziar et al. 2018) Soil remediation techniques and technologies (Wang et al. 2024)
Vectors	Ectoparasites (ticks, fleas, mites, flies, etc.) Mosquitoes	CDC Mosquito Control Resources CDC Tick Data and Resources Tick surveillance programs and related health tools (Eisen and Paddock 2021)

Microbiological detection and quantification methods are typically developed and tested across many laboratories and groups with well-defined method limitations and appropriate quality control practices (see Interstate Technology and Regulatory Council (ITRC) Environmental Molecular Diagnostics (EMD) Section 10 for quality control considerations). These standardized microbial methods usually represent the best technique currently available for the detection and/or quantification of a specific known pathogen (i.e., targeted analysis, which can identify organisms at genera, species, and/or even strain level classifications). To some degree, these standardized methods can be used or modified in some way to capture new groups or subtypes of known pathogens (i.e., suspect screening).

For a BioCEC, reliable and standardized analytical methods may not be readily available, especially for new pathogens that have not been encountered previously (i.e., non-target analysis). Figure 5-2 depicts the transition of analytical methods from targeted screening approaches that are designed to detect a particular organism, to less-specific suspect screening that can help identify the general identity of a new BioCEC based on evidence-developed hypotheses, to non-targeted screenings that can help identify BioCECs that do not fit a known description. These analytical methods and their applications are discussed in detail below in Description of Analytical Methods. Table 5-2 identifies whether the various methods described in Description of Analytical Methods can be used for targeted analysis, suspect screening, and non-targeted analysis, and provides examples for how these methods have been used. Table 5-2 provides a partial list of possible analytical methods and serves as a summary of those methods, which have been highlighted within this document.

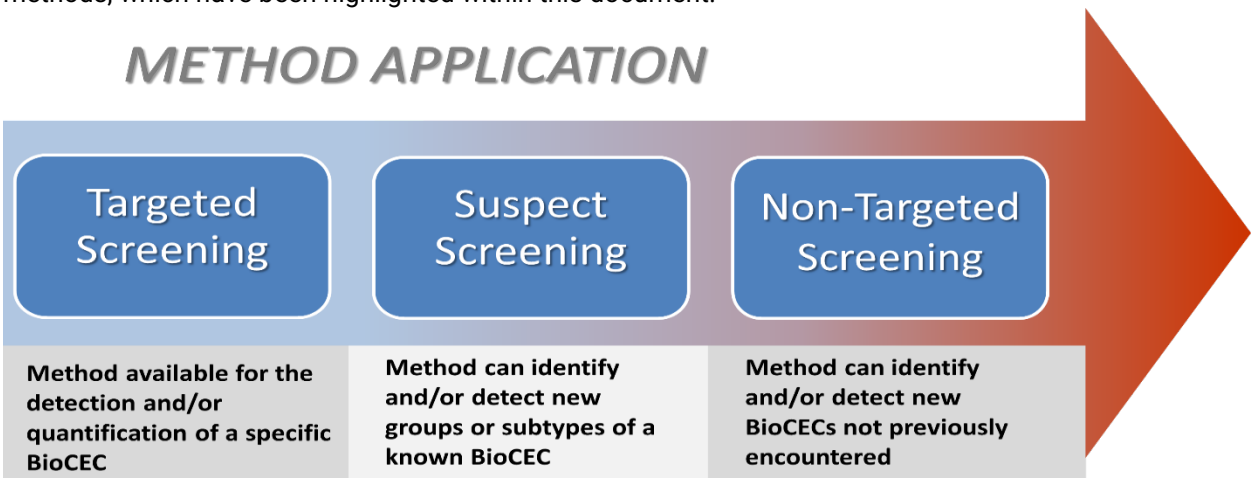

Figure 5-2. Flowchart of the transition from targeted screening to non-target screening of biological contaminants of emerging concern.

Table 5-2. Overview of analytical methods and their applications for the detection of biological contaminants of emerging concern.

Method	Targeted Usage[1]	Suspect Screening[2]	Non-Targeted Usage[3]
Microscopy	Yes. Commonly used in clinical settings for disease diagnosis.	Yes. Fluorescence microscopy is used for viral and bacterial monitoring in diverse marine samples (Noble and Fuhrman 1998). Scanning electron microscopy coupled with energy dispersive x-ray analysis enabled identification and distinction between individual cells of pathogenic microbes (e.g., *Escherichia coli*, *Salmonella typhimurium*, *Listeria monocytogenes*) (Khan et al. 2020).	Yes. Air quality monitoring using microscopy and machine learning can be used to detect particles ≤2.5 µm (Wu et al. 2017).
Culture-Based Methods	Yes (Bonnet et al. 2020).	Yes. Used to detect filamentous fungi in water (Babič et al. 2017), yeasts and fungi from bioaerosols (Kobziar et al. 2018), and selection for tetracycline resistance in soil (Wang et al. 2024).	Maybe. Culture conditions and medium are generally designed to select for specific organisms; however, non-selective agar plates have been used for general capture of culturable organisms in air and water matrices (WHO 2003; Viani et al. 2020).
Flow Cytometry (FC)	Yes (antibodies, fluorescent probes). For targeted analysis, it is often considered appropriate to use FC if fluorescently labeled antibodies against known epitopes for the BioCEC exist or can be prepared, allowing for positive identification of the agent. Example: LITMUS RAPID-B system for detection of bacterial epitopes in food products 16S in situ hybridization with fluorescent probes to identify specific species of bacteria in a sample.	Yes. For suspect screening, FC can assist in identifying key characteristics of organisms. For bacterial screening, gram-staining using fluorophores can be conducted to identify populations within a mixed sample. Additionally, antibiotic resistance of a bacterial BioCEC can be assessed using live/dead fluorescent dye combinations following treatment. Examples: Flow Cytometry Antibiotic Susceptibility Testing (Marutescu 2023); gram-staining and viability staining (Duquenoy et al. 2020).	Yes (general capture/characterization of BioCEC based on size, complexity, etc.). When the BioCEC is completely unknown, FC can be used to identify physical characteristics, notably size, of unknown agents. This can help delineate whether the BioCEC is of fungal, bacterial, or viral origins. Although viruses will often be too small to identify via FC, the absence of larger organisms may lead to the assumption of a viral pathogen.

Method	Targeted Usage[1]	Suspect Screening[2]	Non-Targeted Usage[3]
Matrix-Assisted Laser Desorption Ionization Time-of-Flight (MALDI-TOF) Mass Spectrometry (MS)	Yes. Clinical samples or culture isolates can be rapidly identified or confirmed (Hou et al. 2019; Elbehiry et al. 2022). Can also be used as a rapid diagnostic technique (Feucherolles et al. 2019). Can be used to identify protein toxins (e.g., enterotoxin B), botulinum neurotoxins, Shiga toxin, etc., (Singhal et al. 2015).	Yes. Used for detection of antibiotic-resistance in bacteria (Florio et al. 2020).	Maybe. In development, but heavily dependent on reliability and quality of spectral databases (Ashfaq et al. 2022). Pinar-Méndez et al. (2021) developed a drinking water library of more than 300 bacteria profiles representing 44 new genera and improved identification of water isolates).
Polymerase Chain Reaction (PCR) and Quantitative Polymerase Chain Reaction (qPCR)	Yes. Targeted probes are used for diverse enteric pathogens in human and animal stool, potable water, river water, freshwater, stormwater, and soil (Lappan et al. 2021). Also used for monitoring antibiotic-resistant genes (Franklin et al. 2021; Zhang et al. 2022) and virulence genes (e.g., toxin, adherence, secretion system, iron uptake, etc.) in environmental samples (Xie et al. 2023).	Yes. Used for broadly targeted probes (e.g., 16S, 18S, ITS genomic region). Environmental DNA and RNA can be analyzed by PCR for bacteria, viruses, parasites, fungus, algae, eukaryotes, micro and macroinvertebrates, and vertebrates (Bass et al. 2023).	Yes, general, broad capture probes. Degenerative probes can be used for tracking nitrogen metabolism in diverse microorganisms (Keeley et al. 2020), identifying and differentiating between different SARS-CoV-2 variants (Jessen et al. 2022), and monitoring soil health by targeting bacterial biosynthesis gene domains (Lemetre et al. 2017).
Genomics or Metagenomics	Yes. Provides rapid and precise identification of microbial pathogen species and strains from cultured organisms and environmental samples (Li et al. 2021).	Yes. Culture-independent metagenomic sequencing can be used to identify bacterial strains from an outbreak (Loman et al. 2013).	Yes. Metagenomic sequencing is particularly useful for detecting emerging threats. It provides unbiased detection of all microorganisms present in an environmental sample (Pérez-Cobas et al. 2020). It also provides functional information about the capabilities of microorganisms (Davis et al. 2023).

Method	Targeted Usage[1]	Suspect Screening[2]	Non-Targeted Usage[3]
Fluorescence In Situ Hybridization (FISH)	Yes. Specific RNA and DNA-based probes targeting select pathogens are used. Used for clinical diagnosis of known intracellular pathogens (Prudent and Raoult 2019). Used for rapid and simultaneous identification of respiratory viruses in clinical samples (Hepp et al. 2021).	Yes. Used for broadly targeted probes (e.g., 16S rRNA gene sequences, metabolic enzymes). A modified FISH technique to target and preserve live cells is followed by fluorescence-activated cell sorting to isolate and culture subsets of live bacteria from environmental samples (Batani et al. 2019). Used in the identification of aerobic methane oxidizing bacteria in seawater and sediment samples (Pernthaler and Amann 2004). Used in the monitoring of the *Pseudomonad* genus for soil health (Gougoulias and Shaw 2012).	Yes. Used for broadly targeted probes (e.g., eukaryotic and prokaryotic probes). Used to monitor changes in microbial communities in different sample types such as clinical (Gu et al. 2022) and environmental (Saccà et al. 2019). Saccà et al. (2019) used FISH to monitor bacterioplankton composition in river water and the impacts of industrial chemical exposure.
Microbial Fingerprinting Methods	Yes. Fingerprinting can be used to help identify and confirm a suspected organism, down to strain variation.	Yes. Fingerprinting can be used to help narrow down suspects. Comparing the fingerprint developed to a standard database of microbial fingerprints should allow for the elimination of certain suspects in a pool.	No. Fingerprinting is not advised for non-targeted analysis. Many fingerprinting assays are based around the amplification of particular genes before applying restriction cutting. If the PCR targets are unknown, it is extremely difficult to produce a relevant fingerprint.
Advanced Isothermal Approaches	Yes. Isothermal methods are field deployable and can be used to identify pathogens in environmental samples (Nieuwkerk et al. 2020).	No.	No.

[1] Targeted usage: The best technique currently available for the detection and/or quantification of a specific pathogen. "Yes" indicates that the method is appropriate, or applicable, for targeted usage.
[2] Suspect screening: Standardized methods that can be used or modified in some way to capture new groups or subtypes of known pathogens. "Yes" or "No" indicates whether the method is appropriate, or applicable, for suspect screening.
[3] Non-targeted usage: Application of a method to identify new pathogens that have not been encountered previously. "Yes," "No," or "Maybe" indicates whether the method is appropriate, or applicable, for non-targeted usage.

This section does not discuss the increasing use of data analytics (e.g., machine-learning approaches) for monitoring and forecasting contamination in the environment. For example, Mahmood et al. (2024) address the known data gaps (i.e., missing data) in groundwater quality databases by using two advanced data imputation algorithms. Results suggested that these machine learning–based algorithms can help identify sampling locations, provide geospatial information about contaminants, and prioritize analytes for testing to maximize sampling efforts and efficiently use available, but often limited, sampling resources. Nevertheless, prior to the application of more advanced data analytics, there is a need for reliable detection and quantification of BioCEC through direct analytical methods, which is the focus of this section.

5.1 Description of Analytical Methods

A previous ITRC effort generated detailed descriptions of EMDs, which is a collective term for advanced and emerging techniques for the analysis of biological and chemical characteristics of environmental samples (ITRC 2013). That ITRC EMD webtool and resource provides definitions of various terms also used in this section; they can be accessed by clicking on the word under the **ITRC EMD Glossary** tab. That ITRC resource also contains a detailed appendix of microbiology FAQs providing additional background information if needed (**ITRC EMD Appendix D**). The methods described below can be used for detection and/or quantification of BioCEC in the environmental matrices and subtypes listed in **Table 5-1**. As discussed in this section, selection of an analytical method will depend on numerous factors such as environmental matrix sample type; collection method used; BioCEC characteristics; potential recovery from and concentration in the original sample; and targeted analytical method accuracy, precision, and reliability. Thus, the potential challenges, advantages, disadvantages, and limitations of an analytical method are multifactorial and should be considered throughout the process of designing a sampling and collection plan and choosing an appropriate analytical method.

5.2 Microscopy

Microscopy is a general term used to describe the use of microscopes to view objects at a resolution that cannot be observed with the unaided eye. This method allows for the analysis of shape, size, and other characteristics that allow for the identification and classification of biological samples.

5.2.1 Direct or Light Microscopy

In this method, light is transmitted from a **source** (e.g., lamp) through a condenser, either below or above the sample. The light then passes through the sample to a magnifying lens (or objective), then to the oculars, where the enlarged sample image can be viewed.

5.2.2 Fluorescent Microscopy

This method relies on the underlying process of fluorescence, which occurs when a substance absorbs light of specific wavelengths and emits light at longer wavelengths. Thus, samples are the source of the visible light, in contrast to light microscopy. Most commonly, samples are stained with fluorescently labeled antibodies, nucleic acids, or fluorescent dyes, such as 4',6-diamido-2-phenylindole, propidium iodide, and SYBR green. Samples that autofluorescence can also be detected. In fluorescent microscopy, samples are illuminated with a single or multiple wavelengths of light (excitation wavelength), and the emitted fluorescence (emission wavelengths) reaches the eye or detector (WHO 2005).

5.2.3 Electron Microscopy

This method uses a beam of electrons as the source of illumination to magnify an object, allowing for the visualization of biological structures and composition. Types of electron microscopy include scanning electron microscopy and transmission electron microscopy.

5.3 Culture-Based Methods

Microorganisms can be cultured in either selective or non-selective media under various conditions (e.g., temperatures, atmospheric conditions, incubation times, etc.). The combination of media type and incubation conditions can be used to select for growth of specific microbial groups, subgroups, or species (Bonnet et al. 2020; Lagier et al. 2015). Growth of organisms under these specific conditions may be considered "presumptive," meaning that both the organisms of interest and off-target organisms can grow. In these cases, further testing (using other methods) may be necessary to confirm the identity of the organism. Further sample processing may be required (e.g., filtration, heat, and/or acid treatment) to culture targeted microorganisms. Cell culture systems have also been used to detect, identify, and propagate pathogens (e.g., animal models, embryonated eggs, mammalian cell lines, amoebae) (Vouga and Greub 2016).

5.4 Flow Cytometry

Flow cytometry (FC) is a method that analyzes individual cells or particles in suspension using a flow cytometer (see recent review by Robinson et al. 2023). The flow rate and tubing that draw samples into the cytometer are optimized to allow for a single cell to be analyzed at a time. Analyzers within the flow cytometer use multiple lasers with a variety of wavelengths and angles to investigate an individual cell. From this, the cytometer will collect data on the scatter of visible light by the cell; certain wavelengths can excite fluorescent particles associated with the cell, and the emission from this can be collected by fluorescent detectors. The patterns of the visible light scatter and excitation/emission spectrums of the fluorescence signal can provide important information about the characteristics of the cells or particles (e.g., relative size, internal complexity, surface properties, identity, etc.). To maximize the discriminatory power of this method, samples can also be labeled with fluorescently tagged molecules targeting specific suspects (i.e., fluorescently labeled antibodies) that will allow for specific identification of the biological contaminants.

Method detection limits for FC include size and concentration of the biological agent. If the particles are too small, as is the case with the average virion, the cytometer will likely not be able to detect it. If the agent is too large (i.e., some parasites, or clumps of cells that were not dispersed properly), the detector will not properly categorize the particle. Similarly, the concentration of a target in a sample may also be a limiting factor for detection. If only a few representatives are present in a sample, it may be that any positives could be dismissed as erroneous detections.

A flow cytometer can be equipped with a cell sorter, which will allow for the collection and concentration of cells that meet an investigator's criterion. This can then allow for further investigations using the concentrated sample.

5.5 Matrix-Assisted Laser Desorption Ionization Time-of-Flight (MALDI-TOF) Mass Spectrometry (MS)

MALDI-TOF MS is a method that can rapidly identify pathogens or biological molecules by generating a spectral profile that is then compared against a library of reference spectral profiles of known biologicals (Ashfaq et al. 2022). Profiles are generated by ionizing biological particles (e.g., cellular proteins such as

ribosomes), which move through a flight tube driven by an electric field separating the particles according to their mass and charge. The time-of-flight is measured by instrument detectors at the end of the flight tube. The x-axis of the spectra indicates the mass-to-charge values, and the y-axis shows the intensity of the signal.

5.6 Polymerase Chain Reaction (PCR)

PCR is a method used to amplify specific, targeted DNA sequences. The technique uses a pair of short synthetic DNA segments called primers that recognizes the start (5') and end (3') of the targeted DNA sequence. These primers help guide the enzyme, DNA polymerase, to copy the target DNA sequence through repeated cycles of heating and cooling. This enables the DNA strands to separate and for primers to anneal and then be extended by DNA polymerase to exponentially generate detectable copies of the target DNA sequence (NIH: National Human Genome Research Institute 2025).

5.6.1 Quantitative Polymerase Chain Reaction (qPCR)

This is a very sensitive technique used to amplify short gene sequences (e.g., 80–150 base pairs). It provides real-time monitoring of the exponential amplification process via fluorescence probes or dyes. The detected fluorescence is proportional to the amount of DNA in the reactions, facilitating precise quantification of DNA by interpolation from standard curves. Primers are designed via primer-BLAST (**https://www.ncbi.nlm.nih.gov/tools/primer-blast/index.cgi?GROUP_TARGET=on**) or can be derived from published methods or other peer-reviewed literature.

5.6.2 Digital Polymerase Chain Reaction (dPCR)

This is an advanced nucleic acid quantification method that has no need for standard curves to determine quantities of target DNA. Digital polymerase chain reaction involves partitioning a sample into thousands of small volume reactions, each containing zero or at least one DNA molecule. The number of positive partitions is counted to absolutely determine the exact number of target molecules by using the Poisson mass probability distribution.

5.6.3 High Throughput Polymerase Chain Reaction

High throughput PCR enables the simultaneous amplification and detection of multiple target DNA sequences using a single integrated microfluidic circuit or similar platform (Franklin et al. 2021).

5.6.4 Multiplex Polymerase Chain Reaction

This enables simultaneous detection of multiple DNA targets in a single PCR using distinct primers (ITRC 2013; Ramírez et al. 2015). It provides more information when working with scarce samples.

5.7 Genomics

Genomics is a field of biology focused on studying all the DNA of an organism (i.e., its genome). Sequencing technologies have evolved over the last few decades (Rolando et al. 2024). The first widely used method was developed by Walter Gilbert and Allan Maxam and involved radiolabeled adenosine triphosphate–modified DNA resolved by gel electrophoresis. Frederick Sanger developed first-generation sequencing that used dideoxynucleotides for chain-termination and DNA sequences by gel electrophoresis. Newer sequencing technologies are frequently referred to as next-generation sequencing (NGS). These NGS technologies enable parallel analysis of clinical and environmental samples and are classified into short- and long-read sequencers. The short-read sequencers include Illumina sequencing-

by-synthesis via reversible terminator chemistry, Thermo Fisher Scientific Ion Torrent semiconductor chips, and Roche 454 pyrosequencing (no longer available but mentioned here for historical purposes). The long-read sequencing technologies include PacBio single-molecule real-time and Oxford nanopore electrical current density sequencing.

These NGS technologies are used for genomics, metagenomics, and metatranscriptomics of microbes in environmental samples. Microbial genomics entails bioinformatic assembly of short and/or long reads to generate complete pathogen genomes in pure cultures, which aids their identification (Knight et al. 2018). Metagenomics involves sequencing the entire microbiome in an environmental sample, which yields detailed genomic and taxonomic information for pathogens. Metatranscriptome analysis uses RNA sequences to profile active genes (e.g., **virulence** and antimicrobial resistance genes) to help evaluate microbial activity and discriminate live from dormant or dead pathogens. NGS technologies generate vast amounts of sequence data, necessitating the curation of databases organized for querying and retrieval of information, and phylogenetic and phylogenomic analysis (Vidanagamachchi and Waidyarathna 2024).

5.8 Fluorescence In Situ Hybridization (FISH)

FISH is a method used to visualize and enumerate specific types of microorganisms or groups of microorganisms in an environmental sample (**ITRC EMD**). FISH can provide information regarding the abundance of microorganisms or genes of interest in a sample, cell morphology and growth characteristics, spatial distributions and associations with other microorganisms, and microbial community structure. The FISH method involves (1) the fixation and permeabilization of microorganisms to make their cellular membranes permeable to fluorescently labeled oligonucleotide probes, (2) hybridization of these probes to nucleic acid targets in the microorganisms, (3) washing to remove excess probe, and (4) visualization and enumeration via microscopy or another method, such as FC (**Flow Cytometry**) for high-speed counting.

5.9 Microbial Fingerprinting Methods

5.9.1 Phospholipid Fatty Acid (PLFA)

This method analyzes the key component of microbial cellular membranes and involves several steps, including (1) lipid extraction from the sample, (2) column separation/fractionation, (3) phospholipid modifications, (4) separated and modified components detected by a flame ionization detector, and (5) the generation of a chromatogram profile. PLFA fingerprinting methods provide a measure of total viable biomass and a broad-based profile of the microbial community composition and is best suited for assessing microbial responses as a result of a treatment (e.g., decontamination) or natural or anthropogenic induced environmental changes (ITRC 2013; Lewe et al. 2021; Quideau et al. 2016).

5.9.2 Denaturing Gradient Gel Electrophoresis (DGGE)

DGGE is a non-quantitative technique that provides a DNA-based profile of the microbial community and allows identification of the predominant organisms, generally to the family or genus level. This is achieved by separating PCR-amplified fragments of a targeted gene (e.g., 16S rRNA gene) to allow visualization of patterns of distinguishable bands representing different microorganisms within a sample. DGGE analysis cannot, however, quantify specific organisms or microbial functions present within a sample. This method can also be used to identify and compare the presence/absence of specific organisms among samples. (ITRC 2013).

5.9.3 Pulsed Field Gel Electrophoresis (PFGE)

PFGE is a non-quantitative analysis that allows for the production of a DNA-based profile on genetic material up to 10 megabase pairs in length. This contrasts with traditional gel electrophoresis, which only reliably resolves up to 20 kilobase pairs (Sharma-Kuinkel et al. 2016). By using only specific restriction enzymes to cut the genomic material, specific lengths of genomic material will be generated based on the **host**. These can then be separated using a specialized electrophoresis device that has pairs of electrodes placed at different angles. By changing the angle of the electric field during the run, it effectively gives more distance to resolve the DNA fragments, allowing for the observance of larger bands of DNA. This is ideal for suspect screening methods involving well-characterized suspects, as the DNA band profile that is developed needs to be compared to existing profiles for identification. Additionally, its ability to resolve large genomes lends the technique credence in the identification of bacteria or eukaryotic contaminants.

5.9.4 Multilocus Sequence Typing (MLST)

MLST is a non-quantitative analysis that uses DNA sequencing to determine the identity of an unknown. PCR (**Polymerase Chain Reaction (PCR)**) is performed with primers directed at variable regions within several key genes. These PCR products are then sequenced and compared to existing databases of genomes (or subsets specifically curated for MLST) to determine the identity of the unknown (Larsen et al. 2012). Originally, this was performed using seven key genes, but as the technique has been expanded to include a wider variety of organisms, the exact set of genes to analyze has some variability. This technique has applications for targeted analyses and suspect screening. Although it can be used for non-targeted analysis, it may be more productive to perform whole genome sequencing of an unknown contaminant.

5.9.5 Restriction Fragment Length Polymorphism (RFLP)

RFLP is a non-quantitative analysis that relies on restriction enzymes' ability to cut at specific sites (Hashim and Al-Shuhaib 2019). This method is based on the differences in DNA sequences between BioCEC (e.g., between different strains or subtypes of the same microbial species). For example, DNA sequences for a certain gene may be different between similar BioCEC, but the gene product performs the same function between the similar BioCEC. In this case, these nucleotide differences, or polymorphisms, can be detected when they change where restriction enzymes cut. This is often used in tandem with PCR amplification of particular genes to reduce the background noise of genetic material in a sample. The PCR fragments are then subjected to restriction enzyme cuts, and the size of the resulting fragments is resolved through a technique such as gel electrophoresis. This helps inform the fingerprint of a given sample. This is best used in suspect screening or targeted analysis, as the produced fingerprint needs to be compared to existing fingerprints for identification.

5.9.6 Terminal Restriction Fragment Length Polymorphism (T-RFLP)

T-RFLP is a modification of RFLP that uses PCR primers with fluorescent probes. Rather than analyzing the entirety of the fragments produced by a restriction enzyme cutting a PCR product, only the fluorescently labeled ends of the PCR product (the terminal ends) are analyzed for their change in size (De Vrieze et al. 2018). It effectively reduces the amount of analysis needed to determine differences in the fingerprint, while potentially missing polymorphisms occurring in the middle of the PCR product. The same limitations that apply to RFLP apply here as well.

5.10 Isothermal Amplification Approaches

Isothermal amplification techniques use constant temperature and DNA strand–displacing enzymes that facilitate the extension of gene-specific primers on double-stranded DNA. Exponential amplification of the target sequence is due to isothermal cyclic repetition of these processes and, unlike PCR, does not require thermal denaturation for the primers to bind to template DNA. There are several kinds of isothermal amplification systems, and three are listed below:

Recombinase polymerase amplification (Piepenburg et al. 2006) uses recombinase protein to mediate the invasion of primers into the double-stranded DNA and uses single-stranded binding proteins to stabilize the complex for elongation by an isothermal polymerase such as Phi-29.

Helicase-dependent amplification (Vincent et al. 2004) uses a DNA helicase to enzymatically unwind double-stranded DNA to generate single-stranded complementary strand templates for primer extension by DNA polymerase.

Loop-mediated isothermal amplification (Notomi et al. 2000) involves inner and outer primers that generate a self-priming dumbbell structure with two stem loops that, after multiple rounds of amplification, generate large self-amplifying concatemers (which is a term for a long, continuous DNA molecule that contains multiple copies of the same DNA sequence linked in series).

The advantages of these isothermal amplification approaches are simplicity (constant temperature eliminates the need for thermal cyclers), speed (rapid detection in 30 minutes), and suitability to be deployed in the field (e.g., lateral flow assays). The disadvantage of these approaches is a higher limit of detection relative to PCR.

6 MONITORING PROGRAMS / RESOURCE HUB

6.1 Introduction

The **biological contaminants of emerging concern (BioCEC)** team created an **Excel table** and a narrative explaining how the table was created, its findings, and how these findings can be used to develop BioCEC monitoring programs. The spreadsheet is meant to be useful for both environmental professionals and human health professionals. Monitoring programs highlighted in the spreadsheet can be used as examples for professionals who are interested in formulating their own monitoring programs, as well as for general edification as an overview of the sorts of monitoring programs that already exist within the United States, and to a limited capacity worldwide.

The Excel spreadsheet consists of two tabs. The first tab (Monitoring Programs) shows examples of monitoring programs, with columns specifying the organization initiating the program, the types of biocontaminants monitored, the medium that is being monitored, and links to websites for each of these programs. The second tab (Resource Hub) consists of a resource hub, which includes websites from federal agencies (and others) that have lists of BioCEC and **infectious diseases** that have recently attracted new attention.

The Monitoring Programs tab consists of nine columns identified alphabetically as A through I. The columns provide information on the name of each program (column A), the organization (column B), the state name (column C), specific **pathogen** (column D), health and **environmental media** (columns E and F), a brief discussion of each program (column G), and links to websites pertaining to each program (columns H and I). As noted above, two of the columns identify health and environmental media. The first media column (column E) consists of only those discrete media that are specified in the BioCEC definition spelled out in the **Introduction** section of the BioCEC framework document. The second media column (column F) provides a broader list of media that can be useful to environmental and health professionals trying to identify specific pertinent programs. The team selected monitoring programs and resources from federal agencies, states, and other authorities, including several international organizations. The spreadsheet includes programs focused on environmental media (such as soil, water, and air) as well as programs focused on **vectors** (such as mosquitoes and pathogens transmitted via human-to-human contact and animal-to-human contact). For the state monitoring programs, the team selected programs from one or more states in each region of the United States as defined by US Environmental Protection Agency (USEPA) regional offices. **Methods for Consolidating the Table of Resources and Current Monitoring Methods**, below, provides details on how the monitoring programs were selected.

6.2 Methods for Consolidating the Table of Resources and Current Monitoring Methods

A literature review was conducted to compile a list of state and federal programs responding to/preparing for emerging pathogens in environmental and public health settings. The review applied a common methodology to ensure a compilation of monitoring programs from 2010 to 2024. The review included information from diverse sources, including peer-reviewed literature and non-peer-reviewed gray literature. To capture relevant peer-reviewed articles, searches were performed in established academic databases, including Google scholar, PubMed, Scopus, and Web of Science. Gray literature was sourced from governmental websites, public health organizations, and conference proceedings to identify additional programs and data that may not be represented in traditional academic literature.

The inclusion criteria focused on programs specifically monitoring emerging pathogens within state or federal jurisdictions in the United States, particularly those operating in environmental or public health contexts. Programs were excluded if they targeted non-emerging pathogens, operated solely in international settings without US involvement, or lacked sufficient descriptive detail. A standardized spreadsheet was used for data extraction to ensure consistency and reliability. Key data points included the program's name, the overseeing agency, the specific pathogens monitored, the methods employed for monitoring, the media monitored, and a program description.

The review included 59 monitoring programs that track pathogens. Of these, 22 states were identified primarily by programs managed by state, county, or city level entities, such as state departments of health; several of these states are covered by more than one program in the table. An additional five programs are associated with universities and are likely funded by state or federal agencies. Several examples of monitoring programs from hospitals are also included in the table. The remaining programs were at the federal level (e.g., Centers for Disease Control and Prevention [CDC] or USEPA), had an international focus (e.g., Global Polio Network or World Health Organization), or were broader programs that served multiple areas (e.g., Association of Public Health Laboratories).

The programs reviewed cover an extensive range of pathogens. For instance, four programs focus on polio, while others monitor multiple pathogens, including influenza, *E. coli*, and **vector-borne diseases** like West Nile virus and Eastern equine encephalitis. Some programs address specific needs, such as the Global Polio Laboratory Network's specialized focus on polio eradication or wastewater surveillance efforts that enable early detection of SARS-CoV-2 and other emerging pathogens such as mpox. In some cases, the programs have state or federal mandates to report infectious diseases detected in the human population (i.e., the CDC National Notifiable Disease Surveillance System). Other programs monitor broader categories, such as antimicrobial resistance genes, **zoonotic diseases**, and **communicable diseases** reported under statutory requirements.

These programs use various media for pathogen detection, including water, vector populations (such as mosquitoes), human and animal tissues, and healthcare data systems. The summary sheet reflects the significant role of these monitoring programs in supporting early detection, surveillance, and response to emerging public health threats. The final dataset highlights the breadth of monitoring efforts across the United States and internationally and provides a valuable resource for stakeholders to strengthen public health and environmental protection initiatives.

The list should not be considered exhaustive of all states and jurisdictions that conduct pathogen monitoring. Rather it should be considered exemplary of the types of monitoring programs that could be developed by various state entities.

6.3 Overall Findings from the Systematic Review of BioCEC Programs

Most states generally have programs in place for monitoring biological contaminants in ambient environmental media such as surface water, recreational waters, and drinking water. Additionally, initiatives are in place that encourage states and municipalities to monitor for emerging contaminants in their waters. For example, USEPA's Contaminant Candidate List (CCL) is a list of drinking water contaminants that are known or anticipated to occur in public water systems and are not currently subject to USEPA drinking water regulations. Developing the CCL is the first step in evaluating drinking water contaminants. The most recent CCL proposal, CCL 5, includes 12 microbial contaminants (USEPA 2022a). The USEPA continues to further evaluate contaminants on the list.

The results of the analysis indicate that the most prevalent national programs currently in place, pertaining to monitoring for BioCEC, are focused on detecting pathogens associated with new human

health outbreaks or circulating through human populations. The most commonly used methods include wastewater monitoring, monitoring of human health exposures at public health facilities, and vector monitoring.

The analysis also concluded that there are very few monitoring programs in place for tracking ambient air monitoring. Indoor air monitoring is beyond the scope of this document.

Wastewater at a treatment plant can be a valuable tool for determining whether certain transmittable pathogens are present in a community. Although the BioCEC definition contained in this framework document does not specifically identify wastewater as a separate medium, wastewater monitoring is included in this section due to its value, the prevalence of programs in place for monitoring wastewater, and funding opportunities specific to wastewater treatment. The spreadsheet shows that a number of state and local authorities are monitoring for emerging diseases in their wastewater. For example, programs currently being implemented by the Arizona Department of Health Services, the New York State Department of Health, and Nevada's EMPOWER Program routinely test for pathogens in wastewater such as the viruses COVID-19, mpox, and influenza. These programs help to determine whether specific pathogens are present in the general population. The National Wastewater Surveillance System (NWSS) Utilities Community of Practice provides the opportunity for utilities to share best practices and lessons learned regarding sampling collection logistics, communication with health departments and lab partners, and conveying the value of wastewater surveillance to stakeholders. A link to the **NWSS Community of Practice** is included in the monitoring programs page of the spreadsheet. The NWSS tool allows for rapid assessment of outbreak trends across the country, and additional pathogens are expected to be added to the dashboard in the future (**About CDC's NWSS**).

There are many clinical and public health emerging biological contaminant monitoring programs that could be of interest to environmental BioCEC monitoring programs. For example, New York City routinely tracks emergency room visits, ambulance runs, and pharmacy sales to provide an early warning signal of a possible outbreak. The Arizona Department of Health Services routinely monitors for water, mosquito, and vector-borne diseases in environmental systems such as Lyme disease, Oropouche virus, and hantavirus. Further, because climate change is expected to exacerbate the spread of diseases to new areas, several public health agencies are increasing their monitoring of diseases such as Valley fever, Dengue, and malaria.

The Resource Hub section of the spreadsheet consists of resources from federal and international organizations including the CDC, National Institute of Allergy and Infectious Diseases, World Health Organization, and the USEPA. The material includes lists of emerging diseases, current outbreaks, and funding opportunities for states. Regarding funding opportunities, the Clean Water and Drinking Water State Revolving Fund (SRF) Provisions of Bipartisan Infrastructure Law of 2022 provides $5B through the SRF to reduce exposures to emerging contaminants from public drinking water and wastewater systems. According to the Combined SRF Implementation Memo, the funding is available from 2022 to 2026 (USEPA 2022b, 10) and provides $1B for emerging contaminants in wastewater and $4B for emerging contaminants in drinking water. Biological contaminants and microorganisms such as antibiotic-resistant bacteria, biological materials, and pathogens are among the emerging contaminants covered under these provisions (USEPA 2022b, 36). Information regarding the extent to which funding can be provided to individual monitoring programs, and whether funding will continue as initially planned, is beyond the scope of the Interstate Technology and Regulatory Council BioCEC framework document.

6.4 Opportunities and Challenges to Leveraging Existing Programs to Improve BioCEC Monitoring

Although various monitoring programs have been established by public health agencies, there appears to be a lack of coordination and/or duplication between environmental monitoring programs and public

health–focused monitoring of BioCEC. For example, the Wisconsin Department of Natural Resources, City of Milwaukee Health Department in Wisconsin, and the Michigan Department of Environment, Great Lakes and Energy are all independently monitoring the beaches along Lake Michigan. In some cases, these data are consolidated in one place (i.e., Wisconsin Department of Natural Resources); in other cases, different states are not sharing or reporting data efficiently (i.e., MiEnviro Portal that shows *E. coli* only for the Michigan beach results for Lake Michigan and not the Wisconsin beach results).

Many state and federal programs that are currently engaged in routine water and air quality monitoring programs could be extended to include emerging pathogen monitoring. For example, programs that are currently monitoring wastewater or surface water systems could be expanded using advanced diagnostic tools to detect multiple pathogens simultaneously. Multiplex polymerase chain reaction or metagenomic sequencing of these water samples could be used to detect multiple pathogens within the current monitoring workflows. More details on the monitoring methods and their strengths and weaknesses can be found in the **Analytical Methods** section.

Collaboration and data sharing among different states and federal agencies and incorporation of public-private partnerships could also enhance emerging pathogen monitoring. For example, development of centralized databases that integrate environmental and public health data could improve tracking and analysis of pathogen emergence and spread. Strengthening collaborations with academic institutions, private labs, and non-profits to leverage their expertise and technological innovations could enhance monitoring programs.

Extending existing surveillance programs both geographically and to include alternate media such as wildlife and vector surveillance could improve the sensitivity of monitoring programs by early detection of emerging threats in a state's jurisdiction.

Barriers to expanded monitoring should also be considered as these restraints could limit the adoption of expanded emerging pathogen surveillance. Constraints to expanded monitoring include financial and resource constraints (e.g., laboratory infrastructure, trained personnel); diagnostic limitations for detection of novel pathogens; standardization issues including variability in sampling methods and analytical techniques; barriers to interagency and cross-sector coordination, collaboration, and data sharing; and limited policy or political support for emerging pathogens that may be perceived as low risk. Similarly, some BioCEC are naturally present in the environment at low concentrations. When BioCEC are naturally present, there may not be a threshold value for a BioCEC that indicates some protective action should be undertaken.

Implementation of enhanced monitoring should consider the regional context by tailoring the priorities for detecting the pathogen and considering regional risks, such as mosquito-borne diseases in the southern United States and hantavirus in the western states. These monitoring programs should undergo dynamic assessment and priorities should be regularly reassessed based on emerging data, environmental changes, and pathogen evolution. Finally, the emerging pathogen surveillance should be integrated into a type of One Health framework that monitors human, animal, and environmental health.

The **Monitoring Programs / Resource Hub** spreadsheet is provided as a separate file.

7 CASE STUDIES

7.1 Case Study: Blastomycosis Outbreak

7.1.1 Background

The Process Guide section may be used as a tool to identify a **biological contaminant of emerging concern (BioCEC)** and address potential response actions to take once a BioCEC is identified. This case study on an actual blastomycosis outbreak is used as an example to demonstrate a hypothetical application of the process guide.

7.1.2 Identification and Evaluation of BioCEC

The identification of a BioCEC and its evaluation depends on data collected during monitoring activities for a BioCEC. Questions to ask include the following: Is there an existing monitoring program for the BioCEC of concern? Are analytical methods available and adequate for detecting **pathogens** in various **environmental media**? What kind of response is warranted for the BioCEC? If evidence to support the presence and identity of the BioCEC are available, it is important to develop a site **conceptual exposure model (CEM)** to define the relationships among human **host**, pathogen, environmental factors, and relevant exposure pathways (see the Conceptual Exposure Model section). The generic CEM example presented in Figure 3-2 in subsection 2.4 of the CEM section helps inform decisions and actions necessary to address the BioCEC and its human health effects and disease outbreak control or prevention.

It is important to understand the key variables that may have contributed to the human health effects of the BioCEC and disease development (see the Key Variables section). The eco-epidemiology triangle presented in the Epidemiological Triangle of the Key Variables section highlights the factors that may affect pathogen transmission and disease development with an emphasis on the concepts of ecology in host-agent-environment interactions. See the Key Variables section for a detailed explanation.

7.1.3 Blastomycosis Identification and Evaluation

7.1.3.1 Background Information and Outbreak Identification

Blastomycosis is a lung infection that presents as a community-acquired pneumonia (a pneumonia acquired outside the hospital). It is caused by inhaling spores of the fungus *Blastomyces conidia* that is found in soil. Figure 7-1, which is adapted from material from the Centers for Disease Control and Prevention (CDC) (CDC, n.d.), shows how humans are exposed to the *Blastomyces* mold. The hyphae of the *Blastomyces* mold (1) contain spores (2) that can be inhaled or penetrate the skin (3) of a susceptible human. The spores transform into yeast, which can reside in and colonize the lungs (4) or enter the blood system (5) and be deposited in other body parts.

CDC (2024a) reports that half of infected humans show symptoms of fever, cough, shortness of breath, chest pain, night sweats, fatigue, weight loss, muscle aches, and joint pain. These symptoms are observed from three weeks to three months after breathing in fungal spores. Infected individuals with compromised or weakened immune systems have a higher risk of severe blastomycosis such as pulmonary disease or pneumonia and disseminated infections (disease spread from lungs to skin, bones, joints, and central nervous system). Other risk factors for blastomycosis infection include geography, type of work, hobbies, and race.

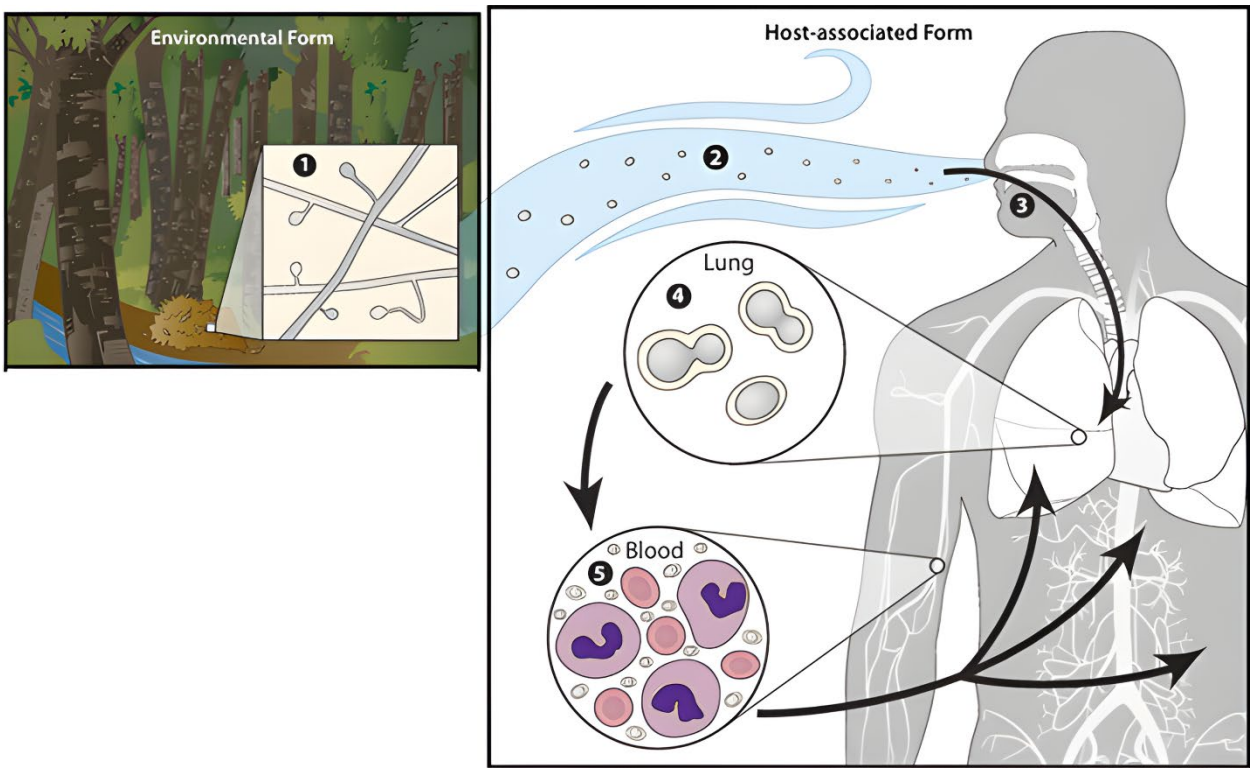

Figure 7-1. Inhalation exposure of a susceptible human host to *Blastomyces*.

Source: Adapted from CDC (n.d.).

CDC (2024c) identifies fungal diseases that are *nationally notifiable* (e.g., coccidioidomycosis) or have *standardized case definitions* (e.g., blastomycosis). The health departments voluntarily submit data to the CDC National Notifiable System only for those classified as nationally notifiable diseases; no reporting is required for diseases classed under case definitions. The case definition is a set of criteria that defines a particular disease. The criteria are used for public health surveillance only and are not recommended for establishing a clinical diagnosis. Several states, including Michigan and Wisconsin, have established blastomycosis as a reportable disease and opted to report data to the CDC.

Figure 7-2 shows areas in the United States that are likely to have *Blastomyces* species in soil, such as Midwestern, South Central, and Southeastern states (CDC 2024a; Seitz et al. 2014). Wisconsin and Minnesota are considered hyperendemic areas. Hyperendemicity indicates a persistently high occurrence of blastomycosis cases. Michigan is considered an endemic area where sporadic blastomycosis in certain areas is observed. Therefore, geography is considered a risk factor for blastomycosis exposure.

The national infection rate for blastomycosis is approximately 2 per 100,000 population with deaths occurring in 8–10% of hospitalized cases. This death rate sharply increased to 17% in 2021; however, this increase may be due to effects of the COVID pandemic, such as a weakened immune response in susceptible populations (e.g., immunocompromised individuals) (CDC 2024a).

CDC (2024a) has indicated that the incidence of blastomycosis may be underreported and therefore lower than what actual numbers may be because testing is limited and disease reporting is not required. CDC (2024 n.d.) has also noted that blastomycosis infection is often misdiagnosed since it presents as community-acquired pneumonia, which makes it hard to differentiate from viral pneumonia (e.g., COVID-19), bacterial pneumonia (e.g., *M. pneumoniae*), atypical bacterial pneumonia (e.g., *Legionella* species) or other fungal pneumonia (*Histoplasma* and *Coccidioides*).

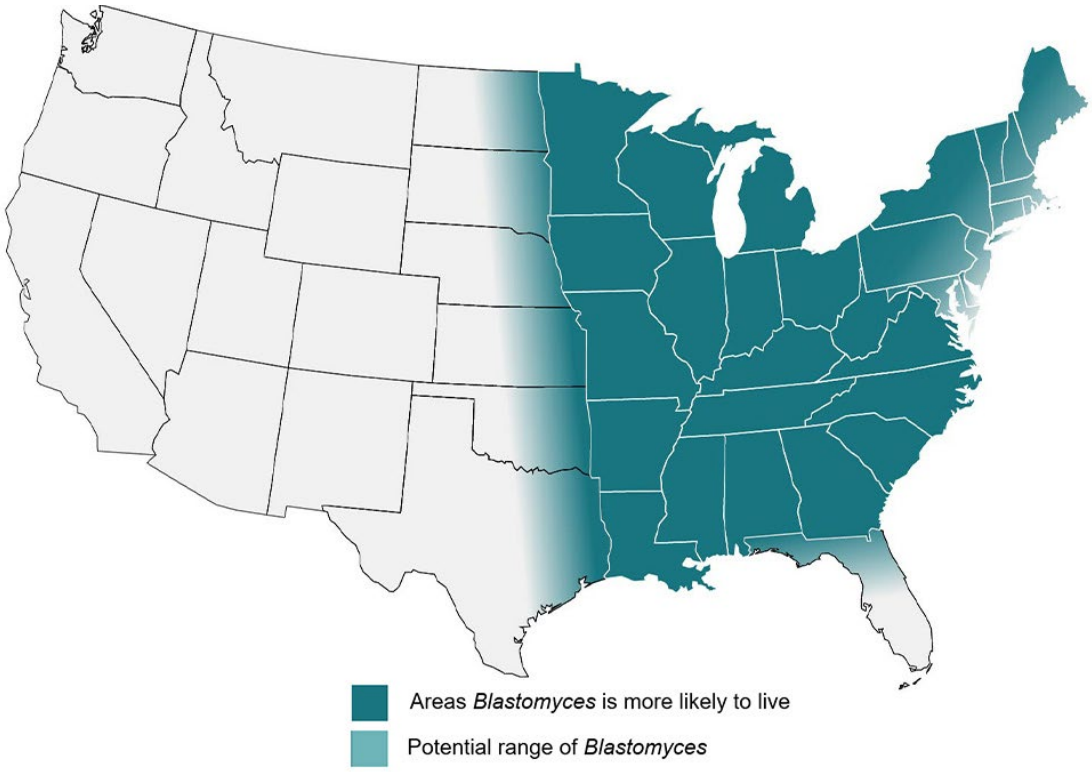

Figure 7-2. Blastomycosis-endemic areas.

Source: CDC (2024a).

Blastomycosis is reportable in Michigan and Wisconsin. In Michigan, the cases averaged less than 30 cases per year as shown in **Figure 7-3**. Delta County, where the blastomycosis outbreak occurred in 2023, averages less than one case per year (MDHHS 2023).

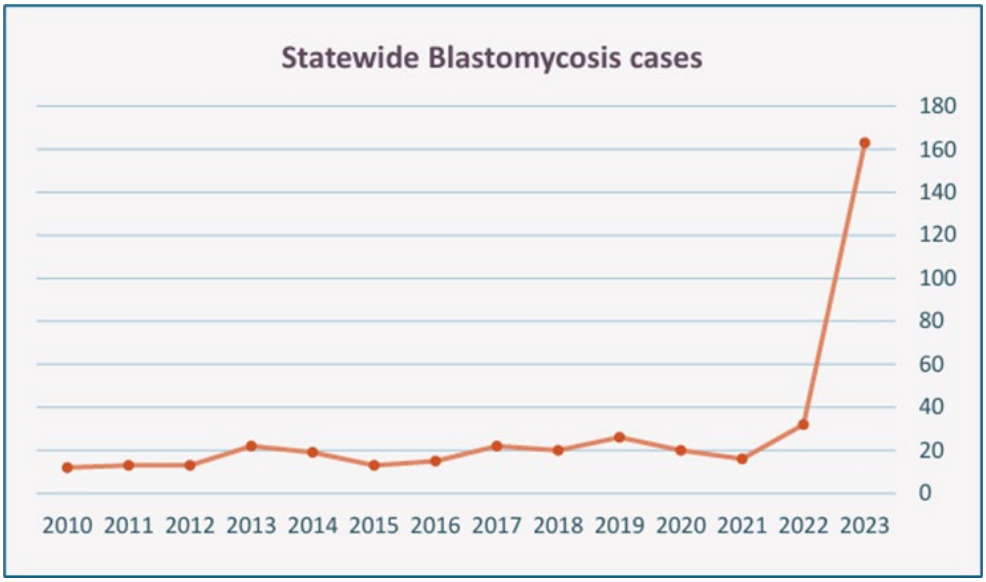

Figure 7-3. Blastomycosis cases in Michigan.

Source: Adapted from the Michigan Department of Health & Human Services (MDHHS) (2023).

Community outbreaks in the United States resulted from soil disruption and outdoor recreational activities in endemic areas (Reik 2024). Previous environmental outbreaks in Wisconsin (from 1984 to 2022) appeared to be associated with excavation, construction, and outdoor recreational activities such as hunting, fishing, or canoeing. CDC (2024b) defines an environmental outbreak as two or more cases of a disease that are linked or attributed to a common **source** or area.

In 2023, the largest documented outbreak of blastomycosis occurred among paper mill workers in Escanaba, Delta County, Michigan. The paper mill uses wood products (logs and chips) to manufacture large rolls of paper (Figure 7-4). Atypical pneumonia infections in male workers of the Escanaba Billerud Paper Mill began in January, and on February 28, the mill management notified the local public health officials of Public Health: Delta and Menominee Counties (PHDM) (Reik 2024). On March 9, PHDM issued a press release indicating that 15 pneumonia cases were being investigated with blastomycosis as the possible cause. The press release included information on blastomycosis including source and symptoms, how it is transmitted, and the incidence in the entire state and the Upper Peninsula region. Prior to the press release, notices were also sent to all local health providers and male mill employees.

Figure 7-4. The Escanaba Paper Mill.

Source: Adapted from Reik (2024).

The Escanaba outbreak was linked to activities that disturbed fungi-infected soil and decomposing organic matter (e.g., plants), including digging or raking leaves, which released microscopic spores that were inhaled by workers. Reported symptoms included fever and cough.

The paper mill asked the National Institute of Occupational Safety and Health (NIOSH) to conduct a human health evaluation of the incident. NIOSH, together with PHDM, the Michigan Department of Health and Human Services, CDC, and area healthcare providers, carried out a collaborative investigation and evaluation of the outbreak. Epidemiologic data collection included employees submitting samples for the urine antigen test and completing a questionnaire on work duties, locations, and health status. A multi-agency site visit in March observed “no single obvious source.”

The characteristics of the Escanaba outbreak were compared to other outbreaks (Table 7-1). The overall occurrence rate in the Escanaba outbreak was 15%. The 89% occurrence in males was higher than those observed in Michigan (54%) and from five other states (64%) during the period 2019–2021 (MDHHS 2023; Williams 2024). The hospitalization rate for the Escanaba cases (12%) was much lower than that for the entire state (45%).

Table 7-1. Comparison of Escanaba outbreak demographics with other outbreaks.

	Outbreak Cases (n=131)	Michigan, 2019–2021	Five US States, 2019–2021
Deaths	1%	8%	14%
Hospitalizations	12%	45%	64%
Age 40–65	57%	50%	45%
% Male	89%	54%	64%

Source: Adapted from Reik (2024).

Certain populations are identified to be at higher risk of blastomycosis, including pregnant women, people with weakened immune systems, and outdoor workers. In addition, people from certain racial and ethnic groups may have higher risk for severe blastomycosis infection or hospitalization (CDC 2024a). In the Escanaba outbreak, workers with blastomycosis were younger and had shorter work tenure at the mill. The median age of workers was 46 years. The epidemiologic investigation included 645 workers. Those with blastomycosis numbered 162 (25%), and the illness was observed from November 2022 through mid-May 2023. No blastomycosis cases were observed in April after the mill was thoroughly cleaned. The prevalence rate among mill workers was approximately 20%. **Figure 7-5** shows the work locations of workers identified as blastomycosis cases.

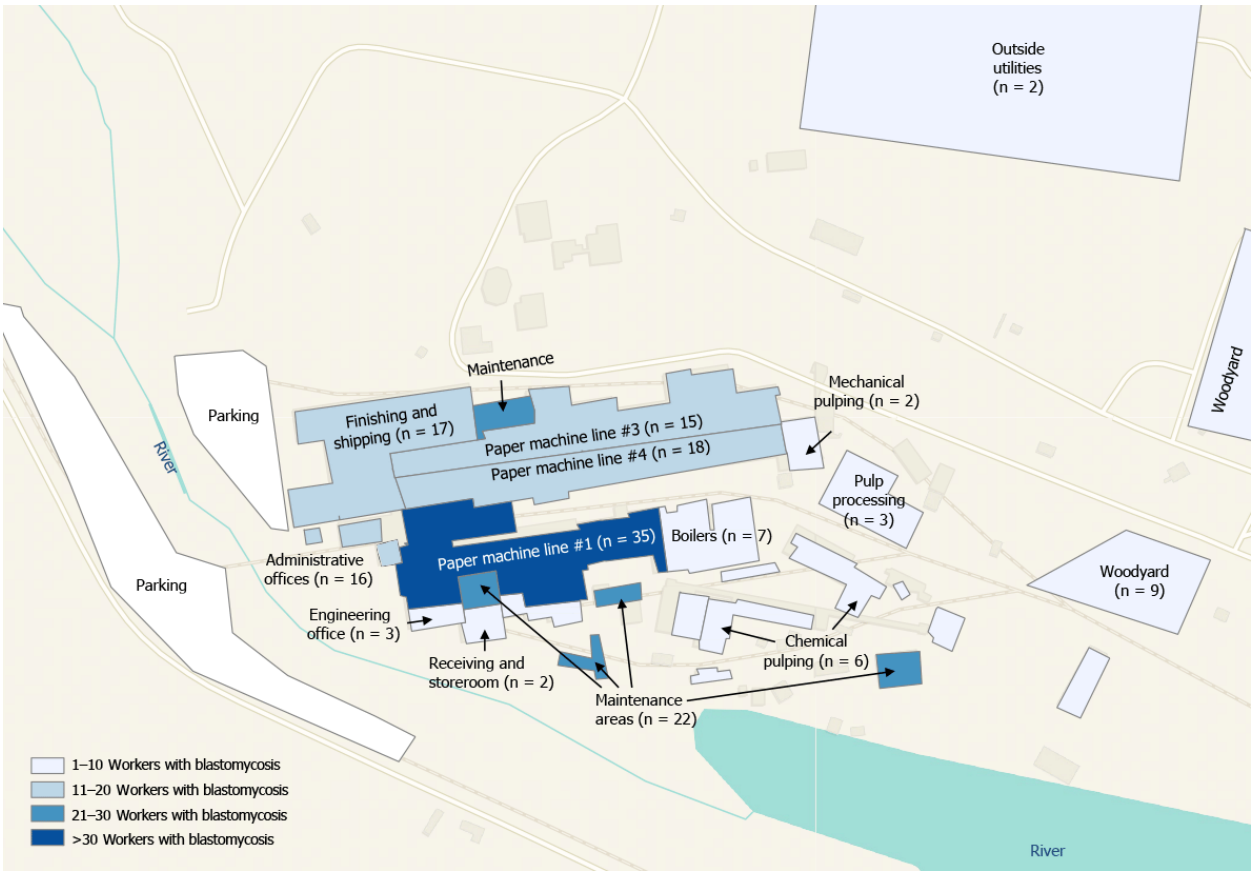

Figure 7-5. Work locations at the paper mill for workers with blastomycosis (n=162) — Michigan 2023.

Source: Adapted from Harvey (2025).

A study of the patients with blastomycosis suggests that although exposures occurred at or near the mill among workers who were young and healthy, those with immunosuppressed conditions, such as diabetes, were the workers who ended up hospitalized (Hennessee et al. 2025). The study reported that prompt testing of the workers and prompt seeking of care by workers experiencing symptoms may have reduced the duration of the outbreak. These factors are attributed to increased patient and provider awareness.

Extensive environmental sampling of various media including soil, wood chips, indoor surface dust and water, dust, duct lining, and HVAC system filters was conducted from various work locations (see **Figure 7-5**) of workers positive for blastomycosis to identify the source of *Blastomyces*. Samples from indoor and outdoor potential sources were analyzed using polymerase chain reaction (PCR) or culture at the Marshfield Clinic Research Institute and University of Wisconsin–Madison research laboratories (Harvey 2025). No *Blastomyces* were detected in those samples.

7.1.3.2 Hypothetical CEM for Blastomycosis Outbreak: (Refer to Figures 7-1 to 7-3)

A hypothetical CEM for the blastomycosis outbreak is presented in **Table 7-2**.

Table 7-2. Conceptual exposure model of the blastomycosis outbreak.

CEM Factors	Key Variables	Description
Impacted Medium	Environmental media in and around the mill	*Blastomyces* living in soil, indoor dust, and other environmental media (wood chips, decaying organic matter, HVAC filters)
Cross-Media Transfer		Release of fungal spores into ambient and indoor air
Exposure Medium	Air	Contaminated indoor and ambient air
Exposure Scenario	Host exposure	Inhalation of fungal spores in air
Exposed Individual	Host	Symptomatic and asymptomatic cases; 50% of exposed individuals become sick
Pathogenicity	Pathogen characteristic	Symptoms are like ones caused by other fungal, viral, and bacterial infections, making diagnosis and treatment difficult; presents as severe blastomycosis — pneumonia and disseminated infections; prevalence rate among mill workers is 20%; death rate among hospitalized cases is 8%–10%
Infected Individual	Host	Susceptibility of immunosuppressed individuals/weakened immune systems

7.1.3.3 Key Variables Considered: (See the Key Variables Section)

BioCEC Pathogenicity and Transmission

Blastomycoses spores in soil can be released into the air due to soil-moving activities or wind erosion. When *Blastomyces* spores are transmitted through the air, it may not be possible to avoid **inhalation** exposure. Infection can cause severe effects such as pneumonia and disseminated infection (extrapulmonary disease) that require hospitalization. The death rate among hospitalized cases is 8%–10%.

The NIOSH health hazard evaluation consisted of epidemiologic investigation (medical survey and urine testing) and environmental assessment of indoor and outdoor environmental samples (indoor surface

dust, HVAC duct lining and filters, water, soil, and wood chips). *Blastomyces* was not detected in the 533 environmental samples using PCR or culture analytical methods. The Michigan Bureau of Laboratories has developed a whole genome sequencing method to speciate the *Blastomyces* species of concern using clinical isolates from blastomycosis cases with positive culture results. Using the whole genome sequencing method, Michigan identified *Blastomyces gilchristii* as the specific BioCEC of concern in the Escanaba outbreak.

Host Exposures

Cases were widespread and not limited to outdoor workers. Cases were identified among office workers and workers with limited duration of exposures (e.g., contractors). Outdoor workers with immuno-suppressed conditions (diabetes, asthma, etc.) had a higher risk of exposure (duration and frequency), as did people with predisposing concomitant disease conditions. CDC (2024a) recommends that people who have weakened immune systems or increased susceptibility to infection should not only avoid activities that involve disrupting soil (yard work, gardening/digging) but also should remain inside during windy or dusty conditions. Nevertheless, most of the mill workers who got sick appear to have been indoor workers.

Environmental Conditions

Fungi are known to live in the soil and organic matter. Soil-related moving activities can cause fungal spores to be released into ambient air. CDC (2024a) reports that certain outdoor activities, such as forestry work, hunting, fishing, camping, or activities that involve digging and excavation, can mobilize more spores into the air and increase the risk of exposure. In addition, high wind gusts can increase spores in the air. In the Escanaba outbreak, blastomycosis cases occurred among indoor workers, specifically those working in the paper machine line #1 (35 cases) and maintenance (22 cases), administrative offices (17 cases), and various other work locations (see **Figure 7-5**). The Escanaba outbreak is an indoor occupational exposure scenario; in contrast, exposure scenarios of other reported outbreaks are related to recreational or soil-related outdoor work.

7.1.4 Actions Taken in Response to the Blastomycosis Outbreak

The **Process Guide** section outlines actions that may be considered when a BioCEC incident or outbreak is confirmed to be present. These include the following actions:

1. *Notifications and coordination with stakeholders and state, federal, and local agencies*
2. *Communication with the public*
3. *Response planning and implementation*

For each action step, key questions are considered such as the following:

- Is the potential BioCEC multijurisdictional?
- How should public communication be coordinated?
- Does guidance for responding to the BioCEC already exist?
- What role should each agency play in public communication?
- How much technical detail should be included in communication?
- What existing CEM could be applied or used to develop a CEM specific to the scenario at hand?

- What is the severity and frequency of risk to public health due to exposure to BioCEC?
- What are the elements of a feasible response to reduce, mitigate, or prevent human exposure?
- What are the roles of various coordinating agencies in the response strategy?

The **Process Guide** section discusses these steps and their key questions.

In the Escanaba blastomycosis outbreak, response actions taken included the following:

- Coordination and collaboration occurred between the company (mill) management, NIOSH, two county health departments, the state Department of Health and Human Services, CDC, and area healthcare providers that provided data to inform the evaluation of the outbreak and implement the response to reduce and eliminate exposures.
- Investigations included collection of information on site and site activities, town hall meetings, worker interviews and urine sampling, extensive environmental sampling of various media in and around the mill, and publication of interim findings.
- Mill management voluntarily stopped operations for three weeks while deep cleaning was undertaken in April. No new cases occurred in May and thereafter.
- Workers wore NIOSH-approved N95 respirators, especially those at higher risk, such as workers with immunosuppressed conditions or those who had more frequent exposures to soil or dust (HVAC filter changing or soil-disturbing activities).
- Information and communication strategies were implemented that included informational town hall meetings to answer employee questions about blastomycosis, their risks and current exposures, and the evaluation process being conducted.
- Communications occurred between the health department and local providers, between mill management and union leaders, and between union leaders and mill workers.
- The involved organizations supported increased media coverage and sharing of local provider experiences with the community.
- Michigan established a website that provides information on blastomycosis, such as a statewide incidence graph and recommended actions, for example, “What can be done by health providers, public health agencies, or individual citizens to protect themselves?”

Successful features of the investigation and response actions include the following:

- Successful and rapid coordination and collaboration by multiple agencies.
- High participation rate in the screening of workers (70% of 800 workers).
- High awareness among workers and the public, which led to faster case identification and treatment.
- Outbreak awareness may have been enhanced by communications that occurred between the health department and local providers, between mill management and union leaders, and between union leaders and mill workers (MDHHS 2023). Additionally, increased media coverage and sharing of local provider experiences with the community may have contributed to the

shortened outbreak duration. CDC (2024a) recommends that information outreach to at-risk people, including those in states adjacent to states where blastomycosis has been reported, may need to be actively conducted by public agencies, health providers, and community leaders.

- PHDM, with help from the CDC and **infectious disease** specialists, developed guidance on how to handle the workers who tested positive on the urine antigen test but were asymptomatic.

- The MDHHS Bureau of Laboratories developed an in-house protocol for whole genome sequencing of isolates, which allowed for speciation and cluster analysis of samples.

Limitations and challenges of the investigation strategies (Harvey 2025; Reik 2024) include the following:

- Use of the urine antigen test for screening a large group of asymptomatic cases. The significance of an asymptomatic person testing positive may be helpful to the investigation but not for clinical evaluation. This test is reported to have a 96% cross-reactivity between blastomycosis and histoplasmosis. In addition, workers who self-reported blastomycosis had taken antifungal medication.

- Difficulty in detecting *Blastomyces* species from environmental samples. There is no established method for testing *Blastomyces* in environmental samples. Culture and PCR at research labs were used.

7.2 Case Study: 2018 *Escherichia coli* Outbreak Linked to Romaine Lettuce

7.2.1 Summary

This case study provides an example of how a CEM can be applied under real-life circumstances. This case study is associated with romaine lettuce grown from the Yuma region of Arizona that was reportedly linked to illnesses in several states and Canada. Five of the 210 reported infected people died. Traceback evidence indicates that the romaine lettuce was from Arizona. The state agencies of agriculture and environmental quality and two federal agencies, the CDC and the US Food and Drug Administration (FDA), coordinated their investigations. The goal was to understand the outbreak including potential causes and environmental transmission pathways, monitoring issues and approaches, stakeholder and community participation, current policies and procedures, and other factors that may have contributed to the outbreak. This case study provides a glimpse of the complexity and the multijurisdictional nature of some BioCEC investigations. The Interstate Technology and Regulatory Council BioCEC guidance provides a review of systematic process and resources for conducting investigations of BioCEC events or outbreaks.

7.2.2 Background

CDC listed 24 foodborne pathogenic outbreaks in 2018. The outbreaks were caused by various **strains** of *Salmonella*, *Vibrio parahaemolyticus*, *Cyclospora*, *Listeria monocytogenes*, and two strains of *Escherichia coli* (*E. coli*), O26 and O157:H7, the last being of concern in this case study. Most outbreaks were traced back to meat, seafood, or vegetables, both cooked and raw, all prepared for shipping and sale for consumption in homes or restaurants. In 2017, one outbreak was associated with leafy greens contaminated with *E. coli* strain O157:H7. In 2019, there were 17 outbreaks, three of which involved the Shiga toxin–producing *E. coli* O157:H7 (STEC) in romaine lettuce or salad kits. STEC is generally associated with cows and is a major cause of food poisoning that can lead to severe complications, including hemolytic uremic syndrome (Alouf et al. 2015).

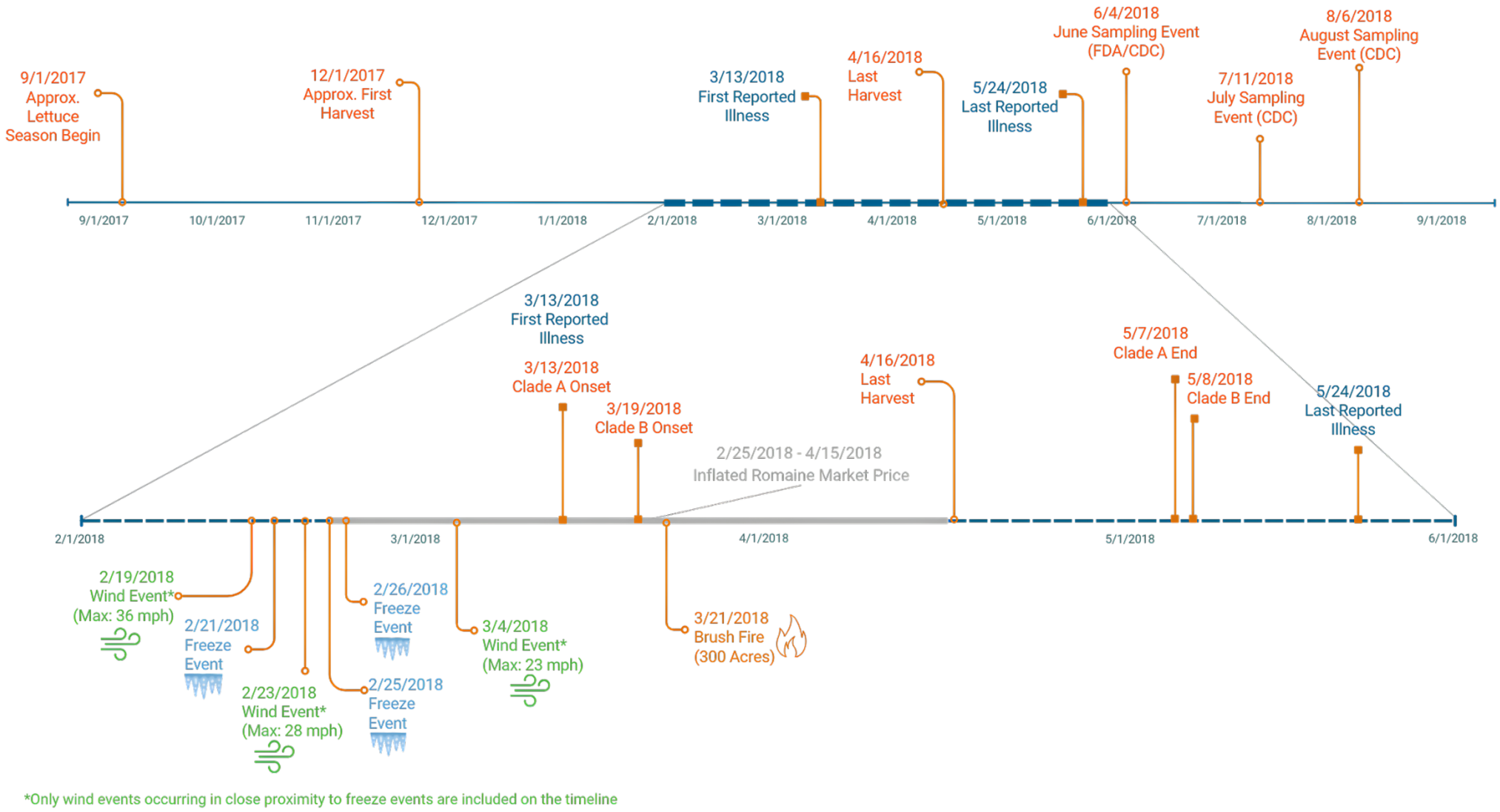

Figure 7-6. 2018 *Escherichia coli* outbreak timeline.

The subject of this case study was associated with romaine lettuce that had been harvested on April 16, 2018. As of June 27, 2018, it was reported that 210 people had been infected in 36 states, and ill people were identified in several Canadian provinces. The infected people ranged in age from 1 to 88 years. Of the 201 people for which information is available, 96 were hospitalized, including 27 people who developed hemolytic uremic syndrome, a type of kidney failure. Five deaths were reported from Arkansas, California, Minnesota (2), and New York.

CDC's summary of this incident may be found in their **web-based archive**. Traceback evidence indicated that romaine lettuce from the Yuma growing region of Arizona was the source of the outbreak. CDC laboratory testing identified a **clade** of *E. coli* O157:H7 in water samples taken from the Wellton Canal. The outbreak is listed as being over as of June 28, 2018.

On July 27, 2018, the Arizona Department of Agriculture (AZDA), CDC, and the FDA requested the Arizona Department of Environmental Quality's (ADEQ's) assistance with determining how O157:H7 was transported to the Wellton-Mohawk Canal. The ADEQ attended **The Leafy Greens Food Safety Task Force** (Task Force) meetings held on July 31 and August 1, 2018, during which the Task Force working groups, CDC and FDA shared their investigation results, observations, and recommendations with the Yuma farming community. Shortly thereafter, on August 6, 2018, a third joint CDC/FDA sampling event was begun and attended by two hydrogeologists from ADEQ. After the August 2018 sampling event, ADEQ assembled an internal team of technical professionals to explore the background factors that influenced the environmental transmission pathways of the outbreak. The timeline of the outbreak event is presented in **Figure 7-6**.

7.2.3 The Growing Fields

Yuma, Arizona, is known as the salad bowl of the nation, producing more than 90% of the winter leafy greens and vegetables consumed in the United States ("University of Arizona Cooperative Extension Newsletter" 2020). Between the months of November and March, Yuma is the epicenter of US production of salad greens.

The agricultural fields in Yuma are interspersed with farmhouses, hobby farms, storage structures, and at least three concentrated animal feeding operations (CAFOs). Cattle are housed in the CAFOs with little to no separation between the fields used for growing lettuces and corn; other feedstock; and the holding pens, drainage channels, detention ponds, and other infrastructure required for operation. The Yuma agricultural fields are located within the Colorado River delta and mark the low point of the division between the Sonoran Desert section and the Salton Trough section of the Basin and Range physiographic province. This area is characterized by low mountains separated by desert plains cut by the flood plains of the Colorado and Gila Rivers (Olmsted et al. 1973).

7.2.4 Watering the Growing Fields

The Wellton-Mohawk Canal transports water from the Colorado River through pumping plants to 58,200 acres of irrigable growing fields in Yuma, Arizona. The Wellton-Mohawk Irrigation and Drainage District has approximately 378 miles of main canals, laterals, and return flow channels (**Figure 7-7**).

Some of these canals have been built below the groundwater table and can gain water under certain conditions. The return water exits the canal system at the Colorado River and includes a network of 90 extraction wells and 300 observation wells used to control the water table and prevent the high saline water from entering the crop root zone. For a more detailed description of this unique water system, please see **https://www.wmidd.org/irrigation.html**.

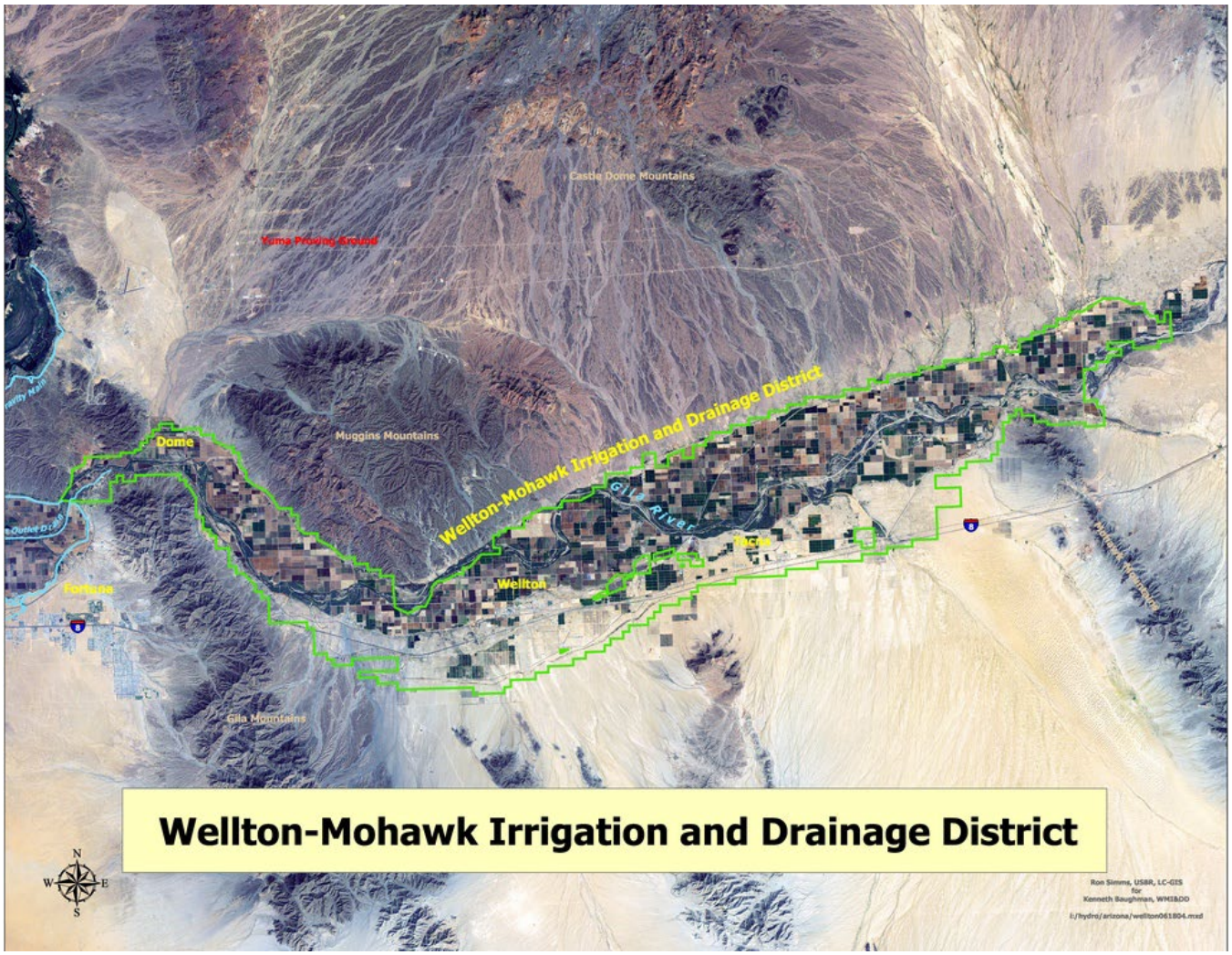

Figure 7-7. Wellton-Mohawk Irrigation and Drainage District.

Source: https://www.wmidd.org/photos.html used with permission.

7.2.5 Conceptual Exposure Model: The Environmental Transmission Pathway

Since human exposure had already occurred, this case study does not discuss the entire CEM because human exposure had unfortunately already come to pass. This investigation was focused on a boots-on-the-ground assessment of potential environmental transmission pathways, many of which still exist today. The CEM includes identification of the following:

- Biological contaminant, in this case *E. coli* strain O157:H7, which produced STEC
- Environmental medium, discussed in **Environmental Medium** below
- **Exposure medium**, the romaine lettuce
- People across North America who ate the romaine lettuce

- Priority, which remains high due to the limited winter season growing areas within the United States and the demand for fresh vegetables in the off season

- Geography, which is unique and mentioned repeatedly throughout this case study

7.2.6 Environmental Medium

Site visits, farmer interviews, and staff knowledge of the farming community and its practices. together with the previous work completed by CDC and FDA, determined potential sources and transmission pathways within the Yuma growing fields. The identification of potential sources and transport pathways was based on the results of three rounds of field sampling completed by CDC and FDA in early June through August 2018. A network map was created to assist in understanding the complexity of potential *E. coli* transport pathways (Figure 7-8) and planning future sampling events. The network depicts all the potential sources of O157:H7 STEC to the Wellton-Mohawk Irrigation and Drainage District and the romaine lettuce growing fields.

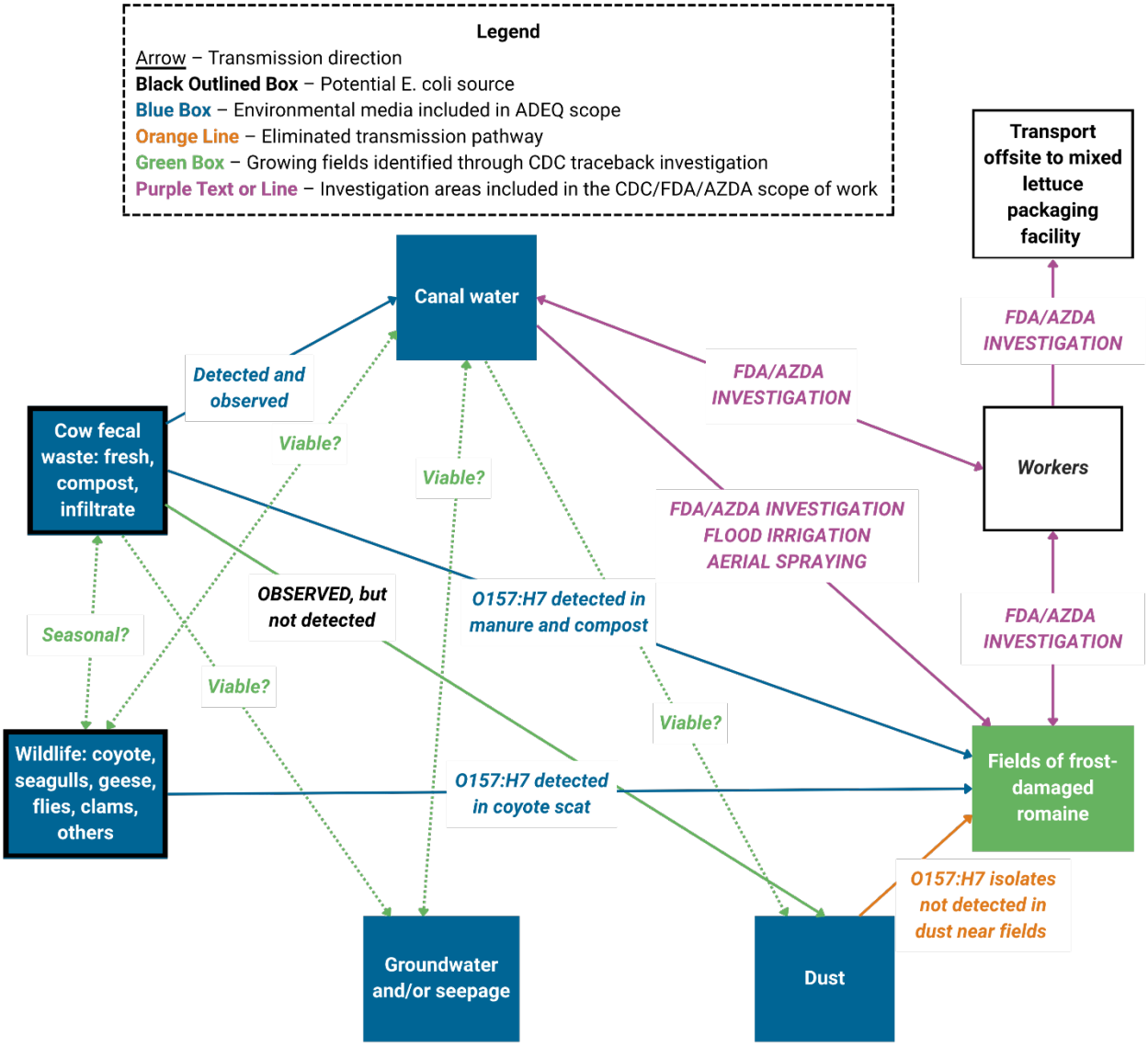

Figure 7-8. Potential transmission pathways.

Note that the sampling conducted in connection with this investigation was limited to a single point in time from specific locations.

The June/July CDC and FDA field results identified the outbreak strain in the following contexts:

- A swab of a cow
- Raw and dry manure and finished compost
- Coyote scat
- Soil along the CAFO fence line
- Sediment within the CAFO
- Algae in the canal
- Filtration to the main delivery canal
- The Welton Field Irrigation Canal
- Canal water near the CAFO
- Upstream of the CAFO within the **irrigation** channel

When mapped, the locations of these samples did not help identify a specific transmission pathway for this outbreak. Although CDC's traceback analysis was able to identify the specific fields from which the lettuce was harvested, the lettuce may have been infected during many activities and in many places, such as during harvest activities, washing the harvested produce, transport of the produce, and packaging the produce into bags of mixed greens. The question remains how and whether STEC was transported to those specific fields and not others and whether there was a common environmental pathway or source to both the canal and the field.

The network diagram illustrates the number of different environmental reservoirs for *E. coli* that exist within the existing agricultural community and infrastructure and the places where life abounds and where we have not studied whether harmful microorganisms can grow within those environments. CDC had discovered that a late frost had damaged the lettuce, making the plant epidermis more conducive to hosting larger bacterial populations. One of the transmission pathway theories identified by the Leafy Greens Task Force involved the potential for *E. coli* to be carried on dust particles that were carried to the fields during major wind events coinciding with the freeze-thaw cycle that occurred in Yuma that winter. This pathway was eliminated because *E. coli* was not detected in any of the dust samples collected in and around the fields.

Another factor that may have affected transmission was the use of canal water for dilution of pesticides that were aerosolized through aerial application by airplane. This theory was evaluated and discarded in a separate investigation conducted by the FDA. Also, many of the identified pathways, when researched, were not found to have any data supporting the viability of *E. coli* under the specific field conditions. For example, there was no data indicating that *E. coli* could survive exposure to the aerobic conditions present in the 45-foot-thick vadose zone, making the viability of that pathway questionable.

After many discussions, literature research, targeted sampling, and information gathering in the field, the team eventually came to the realization that without careful, ongoing monitoring, it would not be possible to evaluate the factors that lead to an outbreak. The numerous septic systems, domestic animals, hobby

farms, CAFOs, itinerant sheep herds, and wild animals all interacting with the canal water at different locations and times would need to be evaluated for the conditions under which an event could result in human exposure to a harmful dose of STEC. Narrowing the root cause down under so many variable conditions would require many data points over time for which ADEQ did not have the funding or capacity to pursue. Due to the lack of funding for the required ongoing monitoring and data evaluation, ADEQ's Director made the decision to halt Yuma-related planning and sampling activities in October 2018.

7.2.7 Root Cause

The Leafy Greens Task Force was convened in July 2018 with a membership of 134 individuals representing growers, shippers, trade associations, state and local government agencies, scientists, consumer advocacy groups, produce buyers, and industry suppliers. The Task Force consisted of five workgroups: 2018 Outbreak Workgroup, CAFO Workgroup, Seasonality Workgroup, Traceability Workgroup, and Communications Workgroup. The FDA and CDC served as technical and informational advisers to the Task Force. The Task Force workgroups reviewed data and information regarding the outbreak and documented recommendations that can be viewed in this factsheet: **About the Leafy Greens Food Safety Task Force (Attachment A)**.

Completion of an Ishikawa Fishbone diagram by the ADEQ team, together with the recommendations of the Task Force, yielded many potential causes that may have contributed to the presence of STEC in romaine lettuce at the time of the 2018 spring harvest. This exercise was chosen and conducted as a way of identifying potential causes of the outbreak so that countermeasures could be identified and preventive measures implemented. Use of root cause exercises for problem solving is part of the Arizona Management System stemming from the practice of the Lean business methodology. The Ishikawa Fishbone diagram is named thus because the commonly used template looks like a fishbone; it is a cause-and-effect exercise that divides potential causes into several categories that stem from the problem or "head" of the fish. In this case, the team was looking for potential causes for the outbreak, such as the following.

- Policies and procedures — Statutes or rules that may have contributed to the problem, how activities were conducted, and whether standards of practice were followed
- People — Human actions, behaviors, skills, or lack thereof
- Measurement — How it is measured, what is measured, and why it is measured
- Environment — The physical, cultural, and perceived factors that may have influenced the situation
- Systems — Software and equipment

The ADEQ team worked to gather as much known information in each of these categories for the analysis, but not all data was easily available. Some of the causes discussed below were discovered as the circumstances unfolded and research progressed. Many of these causes were communicated as recommendations and eventually used to create the Leafy Greens Training Guide available to the farming community through a partnership between CDC and AZDA.

7.2.8 Policies and Procedures

No regulations in Arizona keep farm fields separated from CAFOs, and several CAFOs are located among the Yuma growing fields. Two types of environmental permits may apply to a CAFO: an Aquifer Protection Permit and the Arizona Pollutant Discharge Elimination System Permit (AZPDES). The Aquifer Protection

Permit Nitrogen Management General Permit is required for operation of a CAFO and protects groundwater by minimizing discharge of nitrogen to groundwater from waste impoundments and other CAFO activities through the use of best management practices. An AZPDES permit is required if a facility intends to discharge to a US water body. ADEQs inspection and sampling of the CAFOs showed no detection of STEC on the hides, waste, biosolids, or lagoons and no deficiencies in their practices. Nevertheless, as discussed above, earlier that spring, STEC genetically linked to the outbreak was detected in water and algae samples obtained from the Wellton Canal near the largest closely located CAFO.

Historically, fresh manure and compost is sold to farms in Wellton Valley. Records to track these sales were not obtained as part of this investigation, although STEC was detected in finished compost that spring.

From stakeholder meetings, it was learned that the romaine lettuce harvested in April 2018 was damaged by late frost and was harvested due to increased demand. It was shared that lettuce purchases were made outside of the regular supply chain in order to respond to the greater demand.

7.2.8.1 People

The farming community in Yuma very much cares and wants to prevent public exposure to disease-causing bacteria such as STEC from their produce. They seek to learn and use the most updated agricultural safety practices. That being stated, very little data was available regarding the sanitation practices of farmworkers during the 2018 spring harvest.

7.2.8.2 Measurement

In preparing sampling plans for various environmental media, ADEQ learned that *E. coli* sampling methodologies used by various federal agencies varied depending on field conditions, available equipment, and personnel. It was difficult to find documented sampling procedures for different media, which made it necessary to create those procedures as part of the planning process. Additional updates to Leafy Greens Marketing Agreement metrics included best practices for environmental assessments, specifically the following:

- Climatic and other environmental conditions such as wind speed, direction, and likelihood of frost
- Updating the traceback protocol to be strict in data collection and reporting requirements as opposed to “when available” sampling protocols
- Adoption of the “traceability vision” and best practices from the Global Food Traceability Center

7.2.9 Environment

Many of the environmental factors that arose during the root cause analysis were discussed in **Root Cause** of this case study. Additional factors discussed by the Leafy Greens Food Safety Task Force are listed below:

- STEC can be carried in feces and is commonly associated with cows.
- Wild animals travel unrestricted across the entire area.
- Domestic animals and wildlife live in and around the farms.

- A unique freeze event occurred during the growing season.
- Romaine experiences what is called “epidermal peel” that under specific conditions can make it vulnerable to contamination.
- Bacteria can gain entry to sub-stomata leaf tissues following a freeze event.
- The fields were flood irrigated with Wellton-Mohawk Canal and Gila River water.
- STEC from the same clade was identified in the canal near and upstream of the largest CAFO in the area.
- There were several high wind events, STEC can be aerosolized, and the impacted fields were downwind of the CAFO.

7.2.10 Systems

Systems-related recommendations are listed below:

- Farmers need to obtain and document data on late-season growing conditions and practices.
- Traceback training needed to be created and conducted for key leaders, retailers, and food service workers, and guidance needed to be created for growers operating near CAFOs.
- Traceability workgroup recommendations included description of a “broken system,” where sampling protocols needed to be developed for all stages of product growth and handling.

Scientific research was needed in the following areas:

- Irrigation research specific to the impacted area
- Freeze mitigation strategies for romaine lettuce
- Real-time weather monitoring
- Agricultural water/irrigation treatment
- Air and soil sampling
- Pathogen mitigation technology and strategies
- Dispersion, **deposition**, and *E. coli* survival studies
- Information regarding current industry practices via surveys
- Historical data findings

7.3 Case Study: Quantitative Microbial Risk Assessment (QMRA)

This subsection summarizes three case studies to demonstrate the use of quantitative microbial risk assessment (QMRA) as a tool to prioritize BioCEC and inform decisions. These case studies summarize the approach used to conduct the risk assessment, including hazard identification, dose–response

assessment, exposure assessment, and risk characterization. The World Health Organization (WHO 2025) report, "Quantitative microbial risk assessment: application for water safety management" (WHO 2016) contains an additional three case studies that can be used as an example for those interested in applying QMRA.

7.3.1 Risk of Salmonellosis from Alternatively Produced Broiler Meat (Golden and Mishra 2021)

The case study published by Golden and Mishra (2021) focused on *Salmonella* intervention strategies to mitigate the food safety risks associated with the consumption of contaminated chicken products. *Salmonella* has always presented major risks to the food safety of broiler meat. With the increased popularity of organic meat options, an understanding of the food safety risks associated with these types of products is required.

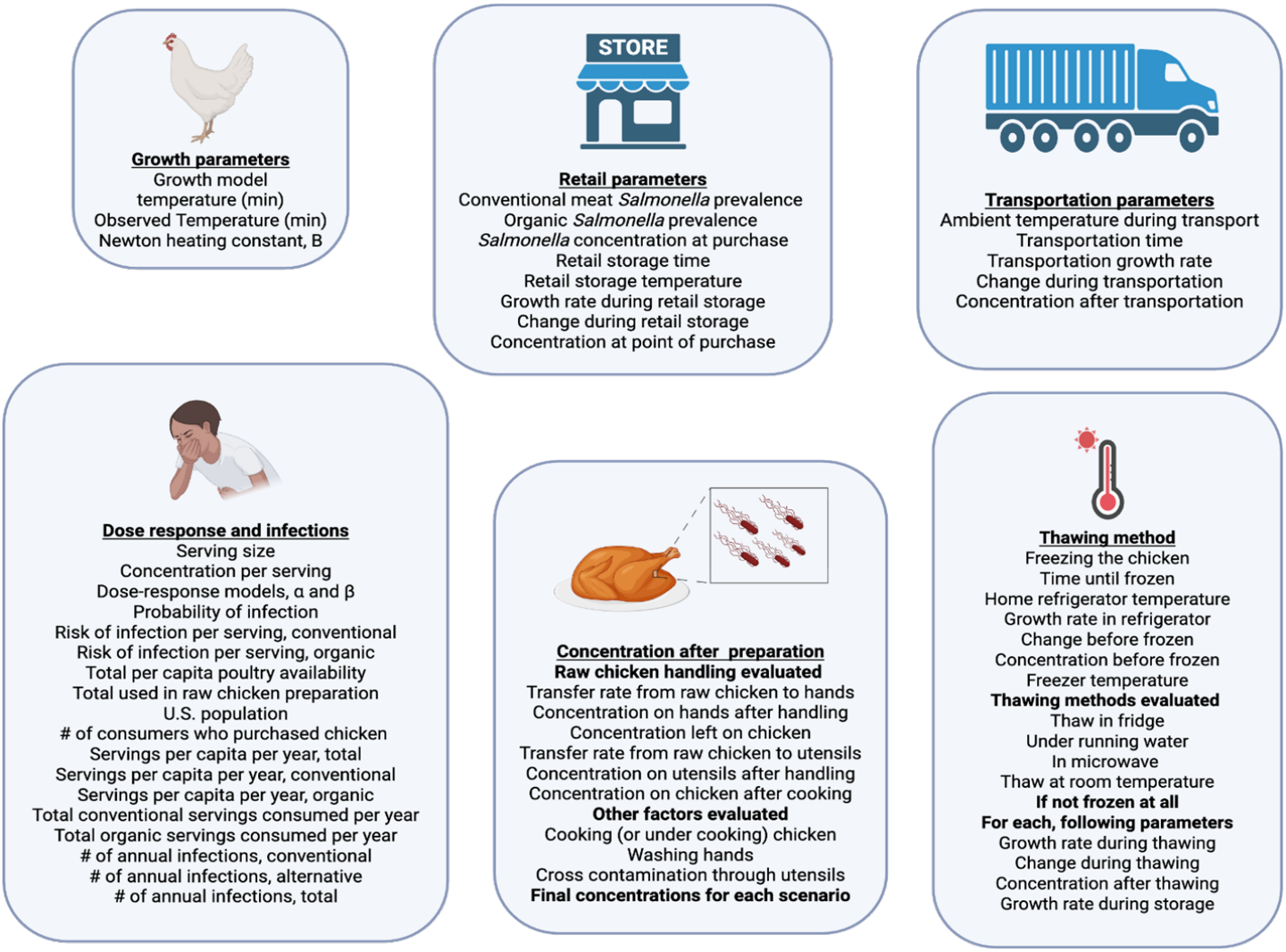

Figure 7-9. Factors incorporated into the quantitative microbial risk assessment performed by Golden and Mishra (2021) to compare the risk of salmonellosis from conventional versus organic meat consumption.

Source: Figure created using data from Golden and Mishra (2021).

The study developed a retail-to-consumption QMRA model that could be used to estimate the differences in risk of salmonellosis acquired from the consumption of conventionally and alternatively (i.e., organic) produced broiler meat in the United States annually. Significant amounts of data were extracted and used to define distributions that could be used to estimate *Salmonella* growth during retail storage, transportation, and home storage, as well as concentration changes during preparation and due to cross-contamination. A Monte Carlo simulation was performed with 100,000 iterations to estimate the risk of infection per serving and total number of infections in the United States annually from both meat types. Sensitivity analyses determined the factors that were highly correlated with increased risk of salmonellosis in both scenarios. QMRA results showed that conventionally produced chicken meat was estimated to have a median risk of infection per serving of 6.4×10^{-8} and cause an average of approximately 3,880,000 infections annually compared with a median risk of infection per serving of 7.7×10^{-8} and an average of approximately 641,000 estimated infections for organic produced chicken. From a risk mitigation perspective, the sensitivity analysis determined that cross-contamination of hands during meal preparation was the most important factor linked to risk. The 'what-if' scenario analysis estimated that using antimicrobial soap during hand washing after handling raw chicken can reduce the risk of transmission considerably (Golden and Mishra 2021). Figure 7-9 highlights the factors that were considered as part of the assessment.

7.3.2 Risk of *Legionella* Infections from Two Shower Exposure Models (Wilson et al. 2022)

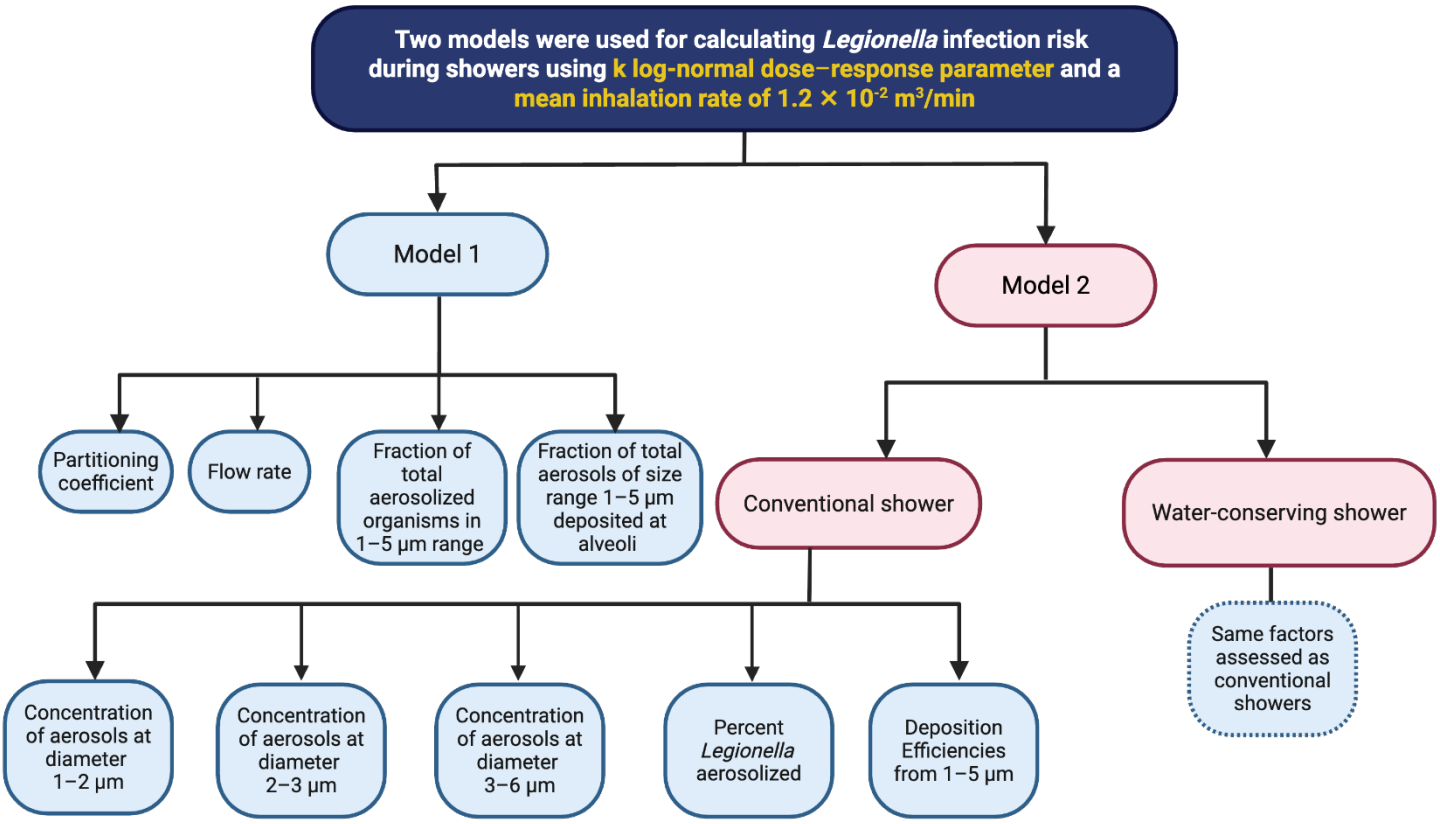

Figure 7-10. Comparing the variables included in the assessment of the risk of *Legionella* infection for a single shower event using two shower *Legionella* exposure models in Wilson et al. (2022).

In this case study, the authors calculate the risk of *Legionella* infection for a single shower event using two shower *Legionella* exposure models (Figure 7-10). The models varied in how they treated partitioning of *Legionella* in aerosols and the aerosol deposition in the lung, with Model 1 using larger and fewer

aerosol ranges than Model 2. In Model 2, conventional vs. water-efficient showers are modeled separately, while Model 1 described exposure for an unspecified shower type (the study did not describe it as conventional or water efficient). A Monte Carlo approach was used to account for variability and uncertainty in these **aerosolization** and deposition parameters, *Legionella* concentrations based on monitoring data, and the dose–response component. Methods for relating infection risks to illness risks accounting for demographic differences were used. Model 2 consistently estimated higher infection risks than Model 1 for the same *Legionella* concentration in water and estimated deposited doses with less variability. When the shower was 7.8-minutes long with a *Legionella* concentration of 0.1 colony-forming units per milliliter (CFU/mL), the average infection risks estimated using Model 2 were 4.8×10^{-6} (standard deviation [SD] = 3.0×10^{-6}) for conventional showers and 2.3×10^{-6} (SD = 1.7×10^{-6}) for water-efficient showers. Average infection risk estimated by Model 1 was 1.1×10^{-6} (SD = 9.7×10^{-7}). The authors concluded that the multiple *Legionella* shower models available for QMRAs yield notably different infection risks for the same environmental microbial concentration.

7.3.3 Using Quantitative Microbial Risk Assessment for Direct Potable Water Reuse Treatment Targets in California (California State Water Resource Control Board 2024)

Direct potable reuse (DPR) is the direct use of recycled water in either a public drinking water system or a raw water supply proximately upstream of a drinking water treatment plant (California State Water Resource Control Board 2024). There is extreme concern for criteria development for DPR to establish the necessary treatment level to safeguard protection of public health. To maintain an acceptable level of human health risk, QMRA is conducted to determine the concentrations of pathogen in finished water. When this information is combined with the initial concentrations of pathogens in raw wastewater, the required $\log_{10}$ reduction values (LRVs) to treat raw wastewater to create finished drinking water is determined (**Figure 7-11**).

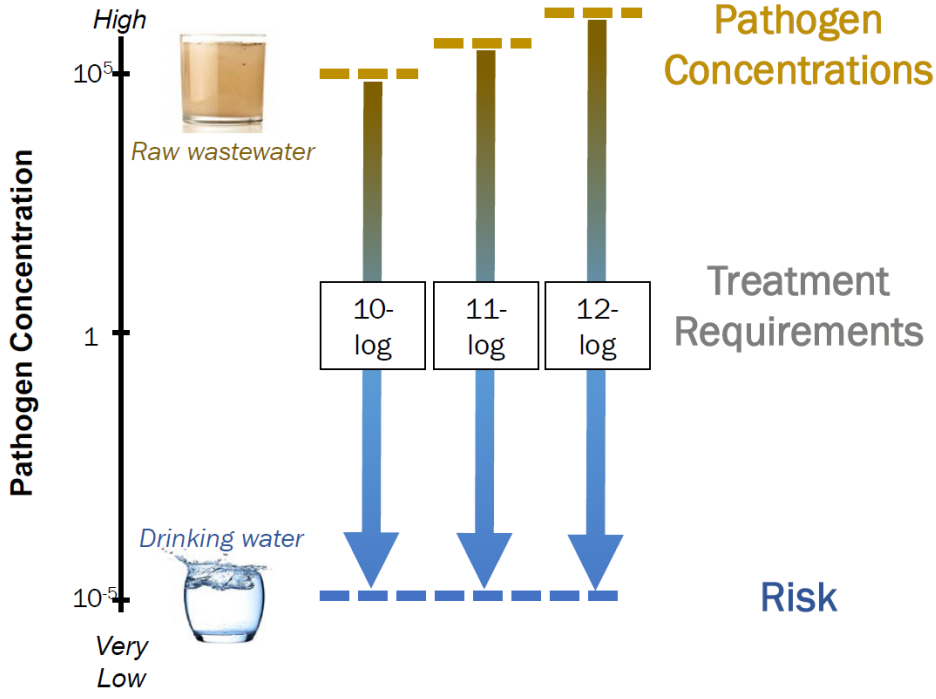

Figure 7-11: Direct potable reuse pathogen risk.

Source: Pecson et al. 2021 © The Water Research Foundation.

A web-based tool called **DPRisk** was created to identify the performance of a specific DPR system and to inform the development of risk-based criteria for the design and operation of DPR systems. The DPRisk tool can be used to estimate how the selection of various log-reduction targets — such as 12/10/10 for enteric virus/Giardia/*Cryptosporidium* — impacts the ability of a system to meet different performance or risk targets, such as the daily risk target of 2.7×10^{-7} infections per person. The DPRisk's main outputs are the resultant distributions of treatment performance and risk analysis (with/without treatment failure) as daily risk and annual risk (Pecson et al. 2021).

California targeted a daily risk of 2.7×10^{-7} instead of 10^{-4} for an annual risk. California set its final required LRVs at 20/14/15 (for enteric virus/Giardia/*Cryptosporidium*) to account for a 6-log treatment failure (lasting 15 minutes) requiring a 4-log treatment redundancy (Pecson et al. 2021).

Other states regulating DPR, such as Colorado and Texas, have required LRVs of 12/10/10 and 8/6/5.5 respectively, for virus/Giardia/*Cryptosporidium* with various justifications and assumptions. Most regulatory QMRA used top-down vs. bottom-up or risk-estimation-focused methods. Top-down QMRAs target the identification of required LRVs based on primary (e.g., raw wastewater) pathogen concentrations and presumed risk goals (Clements et al. 2025).

8 ACKNOWLEDGEMENTS

The members of the Interstate Technology & Regulatory Council (ITRC) Biological Contaminants of Emerging Concern (BioCEC) Team wish to acknowledge the individuals, organizations, and agencies that contributed to this guidance. As part of the broader ITRC effort, the BioCEC Team effort is funded by the U.S. Department of Defense, the U.S. Department of Energy, the U.S. Environmental Protection Agency, and ITRC's Industry Affiliates Program.

The BioCEC Team thanks everyone who participated in the development, review, revision, and completion of the BioCEC guidance and would like to especially recognize the efforts and contributions of the following environmental experts:

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APPENDIX A. GLOSSARY

A

Archaea

Archaea are single-celled microorganisms with a structure similar to bacteria. They are evolutionarily distinct from bacteria and eukaryotes and form the third domain of life. Archaea are obligate anaerobes living in environments low in oxygen (e.g., water, soil, sludge). They were originally defined as extremophiles. Archaea have been associated with various diseases of the human microbiome (e.g., periodontitis, endodontic infections, small intestinal bacterial overgrowth, urogenital tract infections), but they are generally considered non-pathogenic mainly due to our limited knowledge and methodological challenges associated with conducting clinical studies on the inflammatory potential and metabolism of these organisms (Duller and Moissl-Eichinger 2024).

Aerosolization

A contaminant becomes suspended in air in fine soil particulates or in water droplets.

Attenuation

When the virulence of lab strain cultures decreases over time or is completely lost resulting in biased laboratory results on infectious dose and virulence.

B

Biofilms

Biofilms are complex communities of attached microorganisms that are bound to biotic or abiotic surfaces by polysaccharides, proteins, and nucleic acids.

Biological Contaminant of Concern (BioCEC)

A microbial pathogenic agent that may pose newly identified risks to humans through the environment and is found in a vector, water, soil, waste, or air.

C

Capsule

A polymer coat consisting of a dense, well-defined layer surrounding the cell. These structures are not just important for attachment to the host but may also influence interactions with surfaces and attachment to other bacteria.

Clade

A clade is a group of organisms that has a common ancestor. As a result, they often have clade-specific genes in common that confer unique clinical symptoms (e.g., Candida auris, mpox). They are frequently identified using bioinformatics approaches. Understanding biological contaminants of emerging concern

clades can provide insights into where specific strains are concentrated, how they spread geographically, and how to interrupt transmission (Akingbola et al. 2025; Tassy and Fischer 2021).

Communicable Disease

Usually refers to transmission from person to person, or from animal to person (e.g., body fluids, droplets). While all communicable diseases are infectious, not all infectious diseases are communicable (e.g., tetanus is an example of an infectious but not communicable disease).

Conceptual Exposure Model (CEM)

A visual representation of a site, such as illustrations or block diagrams, that maps known and potential interactions among an environment, pathogen, and host. These relationships and interactions presented in the conceptual exposure model inform the identification and evaluation of key variables that influence the presence and severity of a biological contaminants of emerging concern scenario or outbreak.

Cross-Media Transfer

The process of contaminants traveling within and between different environmental media.

D

Deposition

An airborne contaminant lands on a surface.

Dermal Exposure

Contaminated environmental media touches the skin or mucosal membrane of an individual.

E

Endotoxins

Are toxic polysaccharides that are produced as part of the outer layer of the gram-negative bacterial cell envelope. In contrast to exotoxins, which are secreted by living cells, endotoxins are cell bound and are only released upon cell lysis. Endotoxins cause a variety of universal symptoms, such as fever, since the endotoxin causes the release of pyrogens in the host. In addition, endotoxins can cause release of cytokines; diarrhea; and decreased lymphocyte, leukocyte, and platelet numbers. Even though large doses of endotoxin can result in hemorrhagic shock and tissue necrosis, the overall toxicity of endotoxins is lower than that of exotoxins. Studies show that both the lipopolysaccharide and polysaccharide portions are necessary for pathogenicity, since the lipopolysaccharide portion confers toxicity and the polysaccharide portion provides water soluble and immunogenic properties (Madigan et al. 2021).

Environmental Media

Soil, water, and air.

Environmental Persistence

The length of time a pathogen can survive in the environment and retain infectivity.

Epidemiologic Triangle

A model used to describe the interaction among a pathogen, a population susceptible to infection from the pathogen (host), and conditions favorable for exposure of the host to the pathogen (environment).

Exotoxins

Are toxic proteins released from the pathogen cell as it grows. Upon release, the toxins travel to sites away from the site of infection and cause damage. Exotoxins fall into one of three categories: **cytolytic**, **AB toxins** and **superantigen toxins**. Cytolytic toxins result in the degradation of the cytoplasmic membrane and therefore host cell lysis through destruction of phospholipids. Because these cytolytic toxins are usually observed in assays involving red blood cells, these are often called hemolysins. Not all cytolytic enzymes are phospholipases; many affect sterols (e.g., streptolysin O produced by *Streptococcus* spp.) or act as leukocidins that lyse white blood cells and reduce host immunity. AB toxins are composed of two subunits. The B subunit binds to the host cell surface receptor facilitating the transfer of the A subunit across the host cell membrane where it causes damage. An example is the diphtheria toxin produced by *Corynebacterium diphtheriae*, where the A subunit disrupts protein synthesis by blocking transfer of an amino acid from a tRNA to the growing polypeptide chain. The superantigen toxin causes a heightened immune response through increased production of lymphocytes, which cause extensive inflammation and tissue damage (Madigan et al. 2021). **Enterotoxins are exotoxins whose activity specifically impacts the small intestine.** They result in massive secretion of fluids into the intestinal lumen resulting in both vomiting and diarrhea. They are primarily produced by pathogens associated in food poisoning (e.g., *S. aureus*, *Clostridium perfringens*, *Vibrio cholerae*, *Bacillus cereus*, and *Salmonella enteritidis*).

Exposure Medium

An environmental matrix housing the pathogen that a host interacts with.

G

Genotype

The genotype of an organism refers to its genetic properties that may or may not be expressed phenotypically or in a visible manner. Genotyping determines the differences in the genetic makeup of the pathogen by examining the individual organism's DNA or RNA sequences using molecular tools; having a gene does not necessitate its expression or activity. Genes can be up- or downregulated.

H

Host

An organism that harbors a pathogen or parasite.

I

Infectious Disease

An illness caused by the transmission of a pathogen from an infected host to a susceptible host either directly (e.g., person to person) or indirectly (by insects or other animals, or through air, water, food, waste, or soil).

Ingestion

An individual eats or drinks contaminated media.

Inhalation

An individual breathes in an airborne contaminant.

Intake

Water is taken from the environment and used as drinking water, or for industrial and agricultural operations, without complete disinfection.

Irrigation

Surface water, groundwater, or treated wastewater are supplied to land or crops

L

Land-Use Controls

Controls that prevent access to an exposure medium, which can include physical barriers, signage, and restricted use

Leaching

Contaminants within soil or waste become entrained in flowing water and are transported to environmental media on the surface or subsurface

Lethal Dose$_{50}$ (LD_{50})

The LD_{50} is the single dose of an organism, compound, or substance that is expected to kill 50% of a group of test animals in a laboratory setting. The LD_{50} dose is usually expressed as milligrams or grams of material per kilogram of animal body weight (mg/kg or g/kg).

M

Macrophages

Specialized immune cells that engulf and destroy pathogens, debris, and damaged cells; they play a crucial role in innate immunity. They also present antigens to other immune cells, initiating immune responses.

O

Opportunistic Pathogens

Opportunistic or facultative pathogens are organisms for which the host is only one of the potential niches they can exploit to reproduce (Balloux and van Dorp 2017). They usually do not cause disease in healthy hosts and are primarily environmental bacteria, parasites, or fungi that can occasionally cause infection when the right conditions present themselves (Haas et al. 2014).

P

Particle Size

The diameter of the airborne particle.

Pathogen

An organism that can cause disease in a host. The severity of the disease symptoms are referred to as virulence.

Pathogen Invasiveness

The ability of the pathogen to grow in host tissue in such large numbers that it triggers inhibition of host functions.

Pathogenicity

The ability of a pathogen to cause disease in a host.

Phenotype

The expressed (i.e., visible) traits of the organisms, which are influenced by genetic determinants and environmental factors.

S

Slime Layer

Some macromolecules responsible for bacterial attachment are not covalently attached to and secreted by bacteria. This loose network of macromolecules extending outward from the cell is called a slime layer.

Source

A contaminated medium that is primarily responsible for transmitting pathogens to exposure media or susceptible hosts.

Strain

A specific microbial genome or collection of clonally identical cells (i.e., a genotype); one or more colonies (believed to be) derived from the same progenitor cell; or most often, in practice, a collection of cells or genomes within a relatively small range of phylogenetic variation (i.e., a very narrow subspecies clade) (Yan et al., 2020).

T

Toxicity

The ability of the pathogen to cause damage and disease through a preformed toxin that inhibits host cell function, causes damage, or kills the host cell. A good example of this is *Clostridium tetani.* While the bacterium stays at the site of infection, it produces a toxin that moves to different body parts and initiates irreversible muscle contraction and potentially death of the host.

V

Vectors

Organisms that carry a pathogen to a human host.

Vector-Borne Diseases

Diseases that result from an infection transmitted to humans and other animals by blood-feeding arthropods, such as mosquitoes, ticks, and fleas. Examples of vector-borne diseases include Dengue fever, West Nile virus, Lyme disease, and malaria.

Virulence

Severity of disease symptoms.

Z

Zoonotic Diseases

Infectious illnesses that spread between animals and humans. Bacteria, parasites, viruses, fungi, and prions can cause them. Zoonotic diseases can spread through contact with infected body fluids, animal bites, and contaminated water and from eating infected meat. Bats, livestock, rodents, birds, and other vertebrates can carry them.

APPENDIX B. LIST OF ACRONYMS

µm	micrometers
°C	degrees Celsius
°F	degrees Fahrenheit
ADEQ	Arizona Department of Environmental Quality
APHA	American Public Health Association
ArboNET	National arbovirus surveillance system
ASPHL	Alaska State Public Health Laboratory-Anchorage
AWWA	American Water Works Association
AZDA	Arizona Department of Agriculture
BioCEC	biological contaminants of emerging concern
CalEPA	California Environmental Protection Agency
CAFO	concentrated animal feeding operation
CalREDIE	California Reportable Disease Information Exchange
CCL	Contaminant Candidate List
CDC	Centers for Disease Control and Prevention
CEC	Contaminants of Emerging Concern
CEID	Center on Emerging Infectious Diseases
CEIP	California Emerging Infections Program
CEM	conceptual exposure model
CFU	colony forming units
CMR	Code of Massachusetts Regulations
CoE	Center of Excellence
CZVBD	Center for Zoonotic and Vector-borne Diseases
DALYs	disability-adjusted life years
DGGE	denaturing gradient gel electrophoresis

DNA	deoxyribonucleic acid
DNREC	Department of Natural Resources and Environmental Control
DPH	Department of Public Health
DPHL	Division of Public Health Laboratory
DPR	direct potable reuse
E. coli	Escherichia coli
EID	emerging infectious diseases
EIP	Emerging Infections Program
EMD	environmental molecular diagnostic
EMPOWER	Nevada Enabling the Management of Public Health Outcomes through Wastewater Resources
FC	flow cytometry
FDA	Food and Drug Administration
FEMA	Federal Emergency Management Agency
FISH	fluorescence in situ hybridization
FSIS	Food Safety and Inspection Service
g/kg	grams per kilogram
GAMN	General Assessment Monitoring Network
GPLN	Global Polio Laboratory Network
IDB	Infectious Diseases Branch
ITRC	Interstate Technology and Regulatory Council
LAC	Los Angeles County
LD50	lethal dose50
LRV	log10 reduction values
MALDI-TOF MS	matrix-assisted laser desorption ionization time-of-flight mass spectrometry
MassCPR	Massachusetts Consortium on Pathogen Readiness
MD ABCs	Maryland Active Bacterial Core

MDHHS	Michigan Departmen of Health and Human Services
mg/kg	milligrams per kilogram
mg/L	milligrams per liter
mL	milliliter
MLST	multilocus sequence typing
MRSA	methicillin resistant Staphylococcus aureus
NC OSBM	North Carolina Office of State Budget and Management
NDWAC	National Drinking Water Advisory Council
NETEC	National Emerging Special Pathogens Training and Education Center
NGS	next-generation sequencing
NIOSH	National Institute of Occupational Safety and Health
NJDEP	New Jersey Department of Environmental Protection
NNDSS	National Notifiable Diseases Surveillance System
NTU	nephelometric turbidity unit
NWSS	National Wastewater Surveillance System
NYSDOH	New York State Department of Health
PCCL	preliminary Contaminant Candidate List
PCR	polymerase chain reaction
PFGE	pulsed field gel electrophoresis
PHDM	Public Health: Delta and Menominee Counties
PLFA	phospholipid fatty acid
QC	quality control
QMRA	quantitative microbial risk assessment
qPCR	quantitative polymerase chain reaction
Redi-NET	Remote Emerging Disease Intelligence-NETwork
RFLP	restriction fragment length polymorphism
RNA	ribonucleic acid

SD	standard deviation
SRF	State Revolving Fund
STEC	Shiga toxin-producing E. coli
Task Force	Leafy Greens Food Safety Task Force
T-RFLP	terminal restriction fragment length polymorphism
USDA	United States Department of Agriculture
USEPA	United States Environmental Protection Agency
USEPA ORD	United States Environmental Protection Agency Office of Research and Development
USEPA OW	United States Environmental Protection Agency Office of Water
USGS	United States Geological Survey
UV	ultraviolet
VDEQ	Virginia Department of Environmental Quality
WaTCH-WV	Wastewater Testing for Community Health in West Virginia
WBDO	waterborne disease outbreak
WEF	Water Environment Federation
WHO	World Health Organization
WNC	Western North Carolina
WRD	Water Resources Department (City of Asheville, North Carolina)
WSLH	Wisconsin State Lab of Hygiene