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Risk-Based Framework for Developing Microbial Treatment Targets for Water Reuse

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Description of Terms

Disability-adjusted life years	The composite measure of years lost to disability with non-fatal conditions, injuries and diseases, plus the age-specific mortality [years of potential life lost to fatal conditions]. Water guidelines from Australia, Canada and the World Health Organization specify a water exposure annual benchmark of one DALY per million people, which is similar to an annual infection risk of 10^{-3} per person for *Rotavirus* or *Cryptosporidium* spp. and 10^{-4} per person for *Campylobacter* spp.
Fit-for-purpose	Water treated to a quality matching the requirements for the intended use for that water. Appropriate water quality for the intended use is determined based on the agreed level of risk to human health and environmental quality for that use.
Log-reduction credit	The number of credits, expressed as $\log_{10}$ units, assigned to a specific treatment process (*e.g.*, chlorine disinfection, UV oxidation, etc.) for the inactivation of a specific pathogen or group of pathogens.
Log-reduction target	Pathogen control requirement in terms of the $\log_{10}$ (*i.e.*, tenfold) inactivation or removal of pathogens (*e.g.*, 4 log corresponds to 99.99% inactivation/removal).
Log-reduction value	The observed $\log_{10}$ pathogen reduction performance for a unit process operated under controlled and defined conditions. The LRV of a particular unit treatment process is equal to the difference in concentration of an added or endogenous pathogen or surrogate (reported in $\log_{10}$ units) between the influent and effluent of the unit treatment process. This difference may be expressed as a distribution or point estimate (*e.g.*, average value or a selected percentile). A log reduction value crediting framework is required to assign a log reduction value to each unit process in a treatment train.
Quantitative microbial risk assessment	Systematic assessment of the likelihood of negative health consequences, such as infections or illnesses, when a population is exposed to pathogens, which can be used to determine the corresponding treatment level needed to reduce risk to an acceptable level. Follows the process of hazard identification, exposure assessment, dose response, and risk characterization.

Acronyms and Abbreviations

CDC	U.S. Centers for Disease Control and Prevention
CFU	colony forming unit
CSFII	Continuing Survey of Food Intakes by Individuals
DALYs	disability-adjusted life years
DALY | ill	Disability-adjusted life years per case of illness
dPCR	digital polymerase chain reaction
DPR	direct potable reuse
EPA	U.S. Environmental Protection Agency
FIB	fecal indicator bacteria
FoodNet	Foodborne Diseases Active Surveillance Network
FUT2	*fucosyltransferase-2*
GC	genome copies
GC/L	genome copies per liter
GI	norovirus genogroup I
GII	norovirus genogroup II
GWR	groundwater recharge
HBGAs	histo-blood group antigens
IPR	indirect potable reuse
IU	infectious units
L/d	liters per day
LT2 ESWTR	Long Term 2 Enhanced Surface Water Treatment Rule
LRTs	$\log_{10}$ reduction targets
LRV	$\log_{10}$ reduction value
MPN	most probable number
MRA	microbial risk assessment
NHANES	National Health and Nutrition Examination Survey
NRC	National Research Council
PCR	polymerase chain reaction
PFAS	per- and polyfluoroalkyl substances
P(ill|inf)	probability of illness given infection
ppd	per person per day
ppy	per person per year

QMRA	quantitative microbial risk assessment
RNA	ribonucleic acid
qPCR	quantitative polymerase chain reaction
SWTR	Surface Water Treatment Rule
$TCID_{50}$	50% tissue culture infective dose
U.S.	United States
UV	ultraviolet
WHO	World Health Organization
YLD	years of life lived with disability
YLL	years of life lost

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Executive Summary

There are increasing pressures on traditional sources of water throughout the United States, with state and local governments increasingly turning to water reuse to help meet demands. State regulators are tasked with ensuring that recycled water is treated adequately to protect public health in its intended use; this is known as "fit-for-purpose" treatment. EPA's 2012 Water Reuse Guidelines and 2017 Potable Reuse Compendium highlighted the importance of managing acute microbial risks, particularly for applications in which human contact is likely to occur, and discussed quantitative microbial risk assessment (QMRA) approaches to define treatment targets for removing pathogenic viruses, bacteria and protozoa. However, these documents did not provide the detailed scientific information that states, Tribes and other relevant entities need to develop their own risk assessments and microbial treatment targets (log_{10} reduction targets (LRTs)).

This document reviews the state of the science regarding the development of risk-based microbial treatment targets for a range of fit-for-purpose water reuse applications. It articulates the components of the QMRA framework and provides a rationale for input model parameters based on peer-reviewed literature to support states, Tribes and other regulatory entities interested in developing LRTs. The document is designed to provide these entities with sufficient detail to make their own informed decisions regarding the development of microbial treatment targets and provides considerations for decision-makers translating risk-based management into practical implementation.

In addition, the document presents peer-reviewed LRTs for a range of alternative sources of water for both potable and non-potable applications, which were computed using the aforementioned input parameters. The QMRA framework and corresponding LRTs should be viewed as an opportunity to fill important gaps in public health protection to move water reuse forward using scientifically-defensible information. Using the QMRA framework herein, the input parameters and associated LRTs may be updated with new data as they become available to ensure that water reuse treatment decisions are made with the most current science.

This state of the science review covers the following topics:

- A background and introduction to the QMRA framework for water reuse (Chapter 1);
- Information on bacterial, viral and protozoan enteric reference pathogens to consider when evaluating microbial risks related to water reuse (Chapter 2);
- A summary of updated reference pathogen densities in different sources of water and approaches for characterizing those densities to inform water reuse QMRAs (Chapter 3);
- A summary of ingestion estimates for potable reuse, unrestricted irrigation and non-potable indoor uses (Chapter 4);
- An overview of the most current reference pathogen dose-response models and parameter values for determining the probability of infection and illness along with a quantitative characterization of illness severity (Chapter 5);
- A description of risk characterization approaches using both infection and disease burden health metrics (Chapter 6);

- A synopsis of peer-reviewed publications for water reuse treatment requirements (*i.e.*, LRTs) that establish the current state of the science for a wide range of applications (Chapter 7);
- A discussion of key policy considerations for decision-makers implementing risk-based water reuse treatment targets (Chapter 8); and
- Information on research needs specific to the QMRA framework (Chapter 9).

Chapter 1. Introduction—QMRA Framework for Water Reuse

Evaluating potential microbial risks associated with water reuse has been a research topic of interest for several decades (Cooper, 1991; National Research Council, 1998; WateReuse Research Foundation, 2004). Initially, these efforts were focused on evaluating planned potable reuse in locations like the Montebello Forebay (California), the Upper Occoquan Service Authority (Virginia), Denver (Colorado), San Diego (California), Tampa (Florida), Singapore and South Africa (Khan & Roser, 2007). Some of these early risk evaluations were conducted through epidemiological studies (Frerichs, 1984; Nellor et al., 1985; Sinclair et al., 2010; Sloss et al., 1996), as QMRA methodologies were still under development.

QMRA studies follow the general risk assessment approach that the EPA and National Research Council have developed and refined over the last 30 years (National Research Council, 1983, 1994, 2009; U.S. EPA, 1984, 2012b). The QMRA framework comprises four fundamental steps: hazard identification, exposure assessment, dose response and risk characterization (Haas et al., 1999). The EPA's 2012 Water Reuse Guidelines (U.S. EPA, 2012a) and 2017 Potable Reuse Compendium (U.S. EPA, 2017) highlighted the importance of managing acute microbial risks, particularly for reuse applications in which human contact is likely to occur, and discussed using QMRA to define treatment targets for removing pathogenic viruses, bacteria and protozoa. However, previous EPA documents did not provide the detailed scientific information that states, Tribes and other relevant entities need to develop their own risk assessments and microbial treatment targets ($\log_{10}$ reduction targets (LRTs)). This document discusses the necessary QMRA components for developing microbial treatment targets for water reuse (it does not address chemical risks).

One of the first applications of QMRA for potable water reuse was to evaluate the safety of aquifer recharge practices in California (Asano et al., 1992; Tanaka et al., 1998). As interest in potable reuse grew, QMRA (Haas et al., 1999, 2014) was applied to characterize the potential human health risks from exposure to pathogens in a broader range of potable reuse scenarios (Amoueyan et al., 2019; Barker et al., 2013; Chaudhry et al., 2017; Gerrity et al., 2023; Khan & Roser, 2007; Lim et al., 2017; National Research Council, 1998; Pecson et al., 2017; Rose & Gerba, 1991a; Salveson & Soller, 2019; Soller et al., 2018b; Soller et al., 2019; Soller et al., 2017a; U.S. EPA, 2017). These studies used modeling inputs to estimate the probability of infection (a metric of risk) associated with an exposure dose of pathogens from potable reuse (QMRA used in this way is commonly referred as a forward QMRA approach).

Using the same general risk assessment framework, QMRA has also been used to develop microbial treatment targets (LRTs) for both potable and non-potable water reuse systems (Gerrity et al., 2023; Jahne et al., 2024; Pecson et al., 2022a; Reynaert et al., 2024; Schoen et al., 2017; Schoen et al., 2023). In this application, QMRA is used to determine the level of water treatment (*i.e.*, pathogen reduction) that is needed to achieve a specified level of public health protection. This process has previously been referred to as a "reverse QMRA approach" (U.S. EPA, 2014). In an early application of this technique, Regli et al. (1991) computed the mean microbial dosage required to achieve a specified level of risk related to drinking water. For potable reuse, this QMRA approach to develop LRTs is now well accepted (World Health Organization, 2017b), and several states have developed or are in the process of

developing risk-based indirect potable reuse (IPR) and direct potable reuse (DPR) regulations (U.S. EPA, 2023).

Similarly, for non-potable reuse applications, the World Health Organization and the Australian government first estimated non-potable LRTs using QMRA for a limited set of applications and water sources (National Resource Management Ministerial Council et al., 2009; World Health Organization, 2006). States have also begun developing regulatory LRTs for onsite applications using the QMRA approach (U.S. EPA, 2023). Prior to these developments, microbial treatment requirements for onsite non-potable applications were typically based on fecal indicator bacteria (FIB) densities in finished water (*e.g.*, the National Sanitation Foundation/American National Standards Institute, Standard 350-2015 for non-potable onsite reuse of graywater) (NSF International, 2015). However, the FIB-based standards did not correspond to a specific level of human health protection for consumers (National Academy of Sciences, 2016), and it was identified that a quantitative risk approach was needed (National Resource Management Ministerial Council et al., 2009; Schoen & Garland, 2017). This gap was filled with proposed methods for managing onsite reuse systems that explicitly consider pathogen exposure risk through the use of fit-for-purpose LRTs (Schoen et al., 2017; Sharvelle et al., 2017). Subsequently, several publications have derived additional risk-based LRTs for onsite water reuse applications (Jahne et al., 2024; Jahne et al., 2023; Pecson et al., 2022a; Reynaert et al., 2024; Schoen et al., 2023; Schoen et al., 2020a, 2020b).

A systematic literature review on potable reuse risk assessments reported that the assumptions used to conduct QMRAs are critical determinants of the estimated risks (Nappier et al., 2018). Those findings are also relevant to calculating LRTs and generalizable for all water reuse applications, including non-potable water reuse scenarios. The results suggest that the most important aspects of the QMRA framework relate to:

- Reference pathogens included and methods used to enumerate those pathogens (*i.e.*, cell culture methods vs. molecular methods);
- Reference pathogen densities in sources of water and whether statistical distributions or point estimates are used to characterize those densities;
- Reference pathogen dose-response models;
- The source of water used as a starting point for the analysis (*i.e.*, raw sewage vs. secondary effluent);
- Risk characterization approaches and selected health benchmarks (*e.g.*, disability-adjusted life years [DALYs] or infections per person per year [ppy]).

These key QMRA components are examined in this review. Chapter 2 provides information on bacterial, viral and protozoan enteric reference pathogens to consider when evaluating microbial risks related to water reuse. Chapter 3 summaries updated reference pathogen densities in different sources of water and approaches for characterizing those densities to inform water reuse QMRAs. Chapter 4 summaries ingestion estimates for potable reuse, unrestricted irrigation, and non-potable indoor uses. Chapter 5 gives an overview of the most current reference pathogen dose-response models and parameter values for determining the probability of infection and illness, along with a quantitative characterization of illness severity. Chapter 6 describes risk characterization approaches using both infection and disease

burden health metrics. Chapter 7 synopsizes peer-reviewed publications for water reuse treatment requirements (*i.e.*, LRTs) that establish the current state of the science for a wide range of applications. Chapter 8 discusses key policy considerations for decision-makers implementing risk-based water reuse treatment targets and Chapter 9 provides information on research needs specific to the QMRA framework.

Chapter 2. Key Reference Pathogens

The various sources of water that are used for water reuse contain a variable and incompletely known suite of potential human or zoonotic pathogens. This necessitates the use of reference pathogens since it is not practical to account for the presence and density of all possible pathogens in these various sources of water.

The use of reference pathogens in the field of QMRA is an accepted and standard practice (Amoueyan et al., 2017; Chaudhry et al., 2017; Lim et al., 2017; Regli et al., 1991; Roser & Ashbolt, 2007; Schoen et al., 2011; Soller et al., 2017a; Soller et al., 2003; Soller et al., 2010b; U.S. EPA, 2012a). In this context, reference pathogens represent the presence and infectivity of known and unknown members of each relevant microbial group and indicate the type of adverse health effect caused by individual pathogens in those groups (World Health Organization, 2004). For water reuse, treatment emphasis is on the classes of microbes that are transmitted via a fecal-oral route and may cause gastrointestinal illness. These include pathogenic enteric bacteria, enteric viruses and parasitic protozoa. Fungi and helminths remain active areas of QMRA research but are not explicitly considered herein (Hamilton et al., 2024). Opportunistic bacterial pathogens (*e.g.*, *Legionella* spp.) are also not included because they are not primarily transmitted via a fecal-oral route (Armstrong & Haas, 2008; Sharaby et al., 2019) and may proliferate after treatment and disinfection steps. These are controlled by distribution system best practices and assumed to be reduced by prescribed water treatment processes for other microbial contaminants (U.S. EPA, 1989, 2006c).

Individuals and animals who are infected shed enteric pathogens via their feces and other bodily secretions. Therefore, QMRAs that consider water reuse exposures must account for all pathogens that could be present in the source of water used for water reuse (untreated municipal wastewater or other alternative sources of water), not just those known to cause waterborne outbreaks. In this context, reference pathogens should 1) be representative of other waterborne pathogens that are of potential concern (Soller et al., 2010a; Soller et al., 2010b; U.S. EPA, 2014, 2017); 2) be commonly and consistently found in the various sources of water used for water reuse (Gerba & Betancourt, 2019; Gerba et al., 2008; Rose et al., 2004); and 3) have salient peer-reviewed dose-response relationships for ingestion exposure (Fazil, 1996; Haas et al., 1999; Messner & Berger, 2016a; Rose et al., 1991a; Teunis et al., 2016; Teunis et al., 2018; Teunis et al., 2020).

The group of reference pathogens for enteric waterborne QMRA presented in this state-of-the-science framework includes two virus types (norovirus and adenovirus), two groups of protozoa species (*Cryptosporidium* spp. and *Giardia* spp.) and two groups of bacterial species (*Campylobacter* spp. and *Salmonella enterica*). Adenovirus is included in this chapter as a reference pathogen because of its ubiquitous presence in wastewater and its resistance to specific types of treatment. However, as described later, the available dose response information on strains that cause gastrointestinal illness is currently too limited to derive widely applicable LRTs for ingestion exposure during water reuse.

Together, these reference pathogens cause the majority of known gastrointestinal illnesses in the United States (Collier et al., 2021; Gerdes et al., 2023; Hlavsa et al., 2015; Hlavsa et al., 2014; Mead et al., 1999; Scallan et al., 2011). Collier et al. (2021) present an estimate of the burden of all waterborne

diseases in the United States that accounts for underdiagnosis and includes all sources of water and exposure routes. They estimated approximately 33.6 million illnesses in the United States in 2014, with norovirus illness (21.8 million), giardiasis (1.1 million), cryptosporidiosis (0.8 million), campylobacteriosis (1.5 million), and non-typhoid salmonellosis (1.35 million) estimated to be most common illnesses caused by waterborne pathogens via the ingestion route. Collier et al. (2021) used data from active and passive surveillance and other sources from 2000 to 2008 to estimate that 31 major pathogens caused 37.2 million episodes of illness each year in the United States, of which 25.4 million are accounted for by the group of reference pathogens herein. Hlavsa et al. (2015) reported that *Cryptosporidium* spp. was the dominant etiological agent for recreational water–associated outbreaks in the United States and accounted for half of all of the treated recreational water–associated outbreaks reported from 2011 through 2012. Gerdes et al. (2023) reported that norovirus infection was the leading illness associated with drinking water in the United States in 2014.

Using the data and information compiled by the U.S. Centers for Disease Control and Prevention reported by Collier et al. (2021) with the supporting information described above, it may be inferred that the group of reference pathogens outlined above collectively accounts for more than 75% of illnesses from known waterborne pathogens in the United States. Furthermore, based on their physical characteristics and ability to resist water treatment, these reference pathogens have been considered representative of other pathogens of potential concern from the waterborne exposure route (such as other enteric viruses) in QMRA studies (Nappier et al., 2018; Owens et al., 2020; Soller et al., 2010a; Soller et al., 2010b; U.S. EPA, 2014; Zhang et al., 2019). It is noteworthy, however, that depending on the source of water, expected wastewater treatment and end use of the recycled water, not all reference pathogens need to be evaluated in all QMRA studies. For example, Jahne et al. (2023) discussed when and whether bacteria reduction targets should be included in non-potable water reuse scenarios. Soller et al. (2010a; 2010b) suggest that norovirus is the etiologic agent of primary concern in recreational waters impacted by untreated municipal wastewater and treated wastewater effluent when QMRA is coupled with epidemiological data. The following subsections summarize salient aspects of each key reference pathogen for enteric waterborne QMRA.

Bacteria

Pathogenic bacteria are single-celled microorganisms that cause disease. These bacteria can be transmitted through direct contact with an infected host, by ingestion of contaminated food or water or through an intermediate host. Pathogenic bacteria commonly associated with fecal waste—and thus potentially relevant to water reuse—include *Salmonella* spp., *Campylobacter* spp. and pathogenic *Escherichia coli*. Exposures to these pathogenic enteric bacteria generally result in self-limiting, acute gastroenteritis in healthy adults. However, more severe illness outcomes may also occur, such as hemolytic uremic syndrome caused by *E. coli* O157:H7 and long-term sequelae such as Guillain-Barré syndrome associated with *Campylobacter* spp. Because of their relatively large size and cell wall composition, enteric bacteria are generally more susceptible to water and wastewater treatment and disinfection than viruses and protozoa (Nappier et al., 2018).

Campylobacter spp.

Campylobacter spp. are included in the enteric waterborne QMRA reference pathogen list because they are the most common bacterial cause of gastrointestinal illness in the United

States, have a peer-reviewed dose-response relationship that is relevant for water reuse–related exposures, may be transmitted through a waterborne route of exposure and are consistently found in water sources commonly used for reuse (Collier et al., 2021; Soller et al., 2017a; Teunis et al., 2018). The CDC estimates that although most cases are not reported, campylobacteriosis may affect approximately 1.5 million U.S. residents every year (Collier et al., 2021). The Foodborne Diseases Active Surveillance Network (FoodNet) reports that approximately 18 cases are diagnosed annually for every 100,000 people (Collins et al., 2022).

Salmonella enterica. *Salmonella enterica* are included in the QMRA reference pathogen list because they are the second most common bacterial cause of gastrointestinal illness in the United States, have a peer-reviewed dose-response relationship that is relevant for water reuse–related exposures, may be transmitted through a waterborne route of exposure and are consistently found in water sources commonly used for reuse (Fazil, 1996; Haas et al., 1999; Scallan et al., 2011; Soller et al., 2017a). Like *Campylobacter* spp., many cases go undiagnosed or unreported and the CDC estimates that *Salmonella* illness affects approximately 1.35 million U.S. residents every year (Collier et al., 2021). The CDC FoodNet program estimates that approximately 14 cases are diagnosed each year for every 100,000 people (Collins et al., 2022).

Together*, Campylobacter* spp. and *Salmonella enterica* account for over 50% of all estimated annual waterborne bacterial illnesses in the United States (Collier et al., 2021). The next largest contributors to estimated annual illness from pathogens causing waterborne illness are *Shigella* spp., accounting for approximately 450,000 illnesses annually (Collier et al., 2021). However, *Shigella* spp. are not included as reference pathogens in the enteric waterborne QMRA reference pathogen list because illnesses are almost exclusively transmitted via food, person-to-person contact or other non-waterborne routes of exposure (Collier et al., 2021). Additionally, *E. coli* O157:H7, a bacterial pathogen particularly significant in foodborne outbreaks, is not included in the reference pathogen group because their reductions across treatment are similar to *Salmonella enterica* and their density is relatively low in waters commonly used for water reuse, including untreated municipal wastewater (García-Aljaro et al., 2005; Lemarchand & Lebaron, 2003).

Despite their epidemiological importance, bacterial pathogens have been omitted from several state water reuse LRT recommendations because treatment and disinfection of viruses and protozoa are anticipated to be at least equally effective for bacteria (California Water Boards, 2023; Pecson et al., 2022a). Bacteria, because of their larger size and cell-wall composition, are generally more readily removed during filtration and inactivated during disinfection (Jahne et al., 2023; National Research Council, 2012). Nonetheless, enteric bacteria present an exposure risk during water reuse and are relevant to consider during a comprehensive microbial risk assessment, particularly for treatment scenarios in which significant viral and/or protozoan removal may not be required (*i.e.*, rainwater harvesting). The information herein supports the use of *Campylobacter* spp. and *Salmonella enterica* as the key representative bacterial reference pathogens for enteric waterborne QMRA evaluations, which include this pathogen class.

Human Enteric Viruses

A virus is an infectious microbe consisting of nucleic acids surrounded by a protein coat, and in some cases by a phospholipid bilayer membrane known as an envelope. Viruses lacking a membrane (*i.e.*, non-enveloped) are often more persistent in the environment and resistant to treatment disinfectants. A virus cannot replicate alone; instead, it infects cells of a host and uses its components for replication (National Institutes of Health National Human Genome Research Institute, 2023). Viruses that infect and multiply in the gastrointestinal tracts of humans or animals are known as enteric viruses. Most enteric viruses are non-enveloped, as they are resistant to the low pH found in stomach acids. After multiplying, they are excreted in the feces of infected individuals and may enter the environment through contaminated wastewater. Enteric viruses are shed in extremely high numbers in the feces of infected individuals; patients suffering from viral gastroenteritis may excrete up to 10^{11} virus particles per gram of stool (Bosch, 1998).

Human enteric viruses are used in enteric waterborne QMRAs because of their high prevalence and abundance in sources of water commonly used for water reuse, high infectivity potential and documented importance in waterborne disease outbreaks (Jiang et al., 2022). Most waterborne gastrointestinal illnesses are thought to be associated with human enteric viruses (Collier et al., 2021; Scallan et al., 2011). Human enteric viruses of concern in human waste include enteroviruses, hepatoviruses, reoviruses, rotaviruses, adenoviruses, noroviruses and astroviruses (Bosch, 1998; Nappier et al., 2018; U.S. EPA, 2015). Viruses, particularly non-enveloped viruses, are important to water reuse treatment applications because of their small size (0.02 to 0.03 µm), and relative resistance to commonly used disinfectants (Haas et al., 1999; Nappier et al., 2018). Human enteric viruses are present in human fecal matter, wastewater and chlorinated secondary effluent (Eftim et al., 2017; Pouillot et al., 2015), in tertiary effluents (Rose et al., 2004) and in effluents from advanced water treatment facilities (Blatchley et al., 2007; Gerba & Betancourt, 2023; Schmitz et al., 2024; Simmons & Xagoraraki, 2011). The human enteric viruses of interest for water reuse have a limited host range; *e.g.*, to date no animal noroviruses have been detected in human stool (Villabruna et al., 2019). Furthermore, evidence to date suggests that if animal-to-human transmission occurs, such events are likely to be rare and sporadic (Villabruna et al., 2019). Therefore, human enteric viruses are not always included in water reuse QMRAs when human sources of contamination are not of principal concern (*e.g.*, rainwater exposures).

Human enteric viruses are typically detected and quantified using either cell culture or molecular methods. Historically, cell culture has been used to detect and quantify infectious viruses using the total culturable virus quantal assay and the 50% tissue culture infective dose ($TCID_{50}$) assay. However, such methods may underestimate the quantity of infectious viruses by two-fold to 100-fold due to virus diversity and the inefficiencies of sample processing and cell assay replication (Gerba & Betancourt, 2019). Over the past several decades, molecular methods such as quantitative polymerase chain reaction (qPCR) and digital PCR (dPCR) have been developed to more rapidly determine the presence and quantity of specific viral nucleic acids characteristic of chosen targets. Because these methods detect nucleic acids, quantifications capture both infectious and noninfectious genome copies (GCs) (Gerba & Betancourt, 2019; Teunis et al., 2020).

Norovirus. Noroviruses are non-enveloped ribonucleic acid (RNA) viruses. They are included in the enteric waterborne QMRA reference pathogen list because they are the most common viral cause of gastrointestinal illness in the United States, transmitted through a waterborne route of exposure, consistently found in untreated municipal wastewater and water sources used for water reuse in high densities and have a relevant peer-reviewed dose-response relationship. Human noroviruses are now known to be the leading cause of gastroenteritis in the United States and worldwide among persons of all ages (Centers for Disease Control and Prevention, 2008; Collier et al., 2021; Mead et al., 1999; Patel et al., 2009; Scallan et al., 2011; World Health Organization, 2003). They are responsible for more than 50% of acute gastroenteritis outbreaks occurring worldwide each year (Jiang et al., 2022). In the United States, Collier et al. (2021) estimated that there are 33.6 million illnesses annually from pathogens that cause waterborne illness, and that norovirus is responsible for approximately 22 million of those illnesses—more than any other known pathogen and more than all other known pathogens combined. Similarly, Hall et al. (2013) estimated that noroviruses are responsible for 19 to 21 million total illnesses each year in the United States, as well as 570 to 800 deaths.

Norovirus has become a key component in the majority of the water reuse risk analyses published since 2010 (Barker et al., 2013; Chaudhry et al., 2017; Lim et al., 2017; National Research Council, 2012; Schoen et al., 2017; Schoen & Garland, 2017; Soller et al., 2018b; Soller et al., 2017a). Researchers have been concerned about norovirus densities in untreated municipal wastewater (measured in genome copies or GCs) corresponding to infectious virus particles because the ratio of GCs to infectious virus particles varies widely for other types of viruses in wastewater for which conventional cultivation methods are available (National Water Research Institute, 2021; WateReuse Research Foundation, 2021). However, a meta-analysis of clinical trial and oyster outbreak data demonstrates that norovirus genomes from harvested oysters impacted by untreated/poorly treated sewage remain highly infectious (Le Guyader et al., 2006; Le Guyader et al., 2008; Teunis et al., 2020), specifically norovirus genogroup I (GI) and norovirus genogroup II (GII), which are most commonly associated with human infections (Koo et al., 2010; Patel et al., 2009). The analysis estimates that in individuals who are susceptible to infection the mean risk of infection of norovirus is 28% when exposed to 1 GC of norovirus GI and 7.6% for 1 GC of norovirus GII. Individuals with a functional *fucosyltransferase-2* (FUT2) gene have histo-blood group antigens (HBGAs) present in the mucosa of the gut and are known as “secretors.” Susceptibility to norovirus infection is strongly associated with secretor status, with non-secretors being resistant to some norovirus genotypes (Johnson et al., 2022).

Although noroviruses continue to be difficult to culture (Ettayebi et al., 2021; Papafragkou et al., 2014), and challenges remain in understanding their rate of inactivation through treatment and disinfection processes (Ryu et al., 2021), it has been suggested that the best approach at present is to use molecular methods (*i.e.*, PCR) to assess the density of enteric viruses in untreated wastewater, where most viruses may be expected to be infectious (Gerba & Betancourt, 2019). Additionally, molecular measurements were used to characterize received doses in the challenge and outbreak studies that inform existing does-response models. McBride et al. (2013) suggest that this kind of “dose harmonization” may reduce uncertainty and increase confidence in the applicability of the published dose-response relationship for risk assessments.

Prior to the publication of the first dose-response relationship for norovirus in 2008 (Teunis et al., 2008), viral risk assessment typically involved the use of culturable virus data, such as for enteroviruses or total culturable viruses, and an available viral dose-response relationship for a representative type of enteric virus. For example, Asano et al. (1992) evaluated the early California wastewater reclamation criteria using enteric virus monitoring data (culturable virus data from unchlorinated secondary effluents) coupled with dose-response data for echovirus and poliovirus reported by Haas et al. (1983). Regli et al. (1991) generalized this approach by suggesting the use of a combination of characteristics of different viruses to compute appropriate levels of treatment for protecting the public from the exposure to viruses in drinking water. For example, they suggested enteroviruses (measured by cell culture) could serve as an indicator of potential occurrence of any relevant virus and be coupled with the dose-response model for the most infectious virus known at the time (*i.e.*, rotavirus). There are numerous examples of this general approach in the literature (Rose & Gerba, 1991b; Tanaka et al., 1998; U.S. EPA, 1989), and it continues to be used and reported today (National Water Research Institute, 2021; Pecson et al., 2022a). The benefit of pairing culturable virus data with the rotavirus dose-response curve was confidence that the measured viruses were infectious (Pecson et al., 2023).

However, scientific advances over the last several decades, particularly related to the importance of noroviruses (Collier et al., 2021) and their presence and infectivity in untreated municipal wastewater/sewage (Le Guyader et al., 2006; Le Guyader et al., 2008; Teunis et al., 2020), provides renewed impetus to reevaluate the limitations of the use of culturable viruses as viral reference pathogens. Examples of limitations include: 1) total culturable viruses and/or culturable enteroviruses account for an unknown and likely small portion of viral enteric illnesses (Gerba & Betancourt, 2019; Gerba et al., 2017; Gerba et al., 2018; Papafragkou et al., 2014); 2) not all viruses will form plaques or may require mixed cell types or pretreatment of cells before inoculation to form plaques (Benton & Hurst, 1986; Gerba & Betancourt, 2019); and 3) advances in molecular biology that allow for the detection of the viral genome known to infect humans and animals in environmental samples have revealed that the number of viruses may be 100 to 1,000 times greater than that detected by cell culture (Gerba & Betancourt, 2019). These limitations also apply to the use of culturable enterovirus as a reference pathogen for enteric waterborne QMRA. For example, the most common viruses in wastewater detected by cell culture are enteroviruses or reoviruses (Gerba et al., 2017). However, it is unclear how many illnesses are caused by the viruses captured through culture assays relative to the estimated viral illnesses reported in epidemiological observations (Gerba & Betancourt, 2019; Gerba et al., 2017; Gerba et al., 2018; Papafragkou et al., 2014). Together, this information suggests that the use of culturable virus data likely underestimates the occurrence and density of enteric viruses in environmental samples (Gerba & Betancourt, 2019).

Adenovirus. Adenoviruses are discussed here because they are a common viral cause of illness in the United States, may be transmitted through a waterborne route of exposure, are consistently found in sources of water used for water reuse in high densities and are resistant to treatment disinfectants (Jiang, 2006).

Adenoviruses typically cause mild infections involving the upper or lower respiratory tract, gastrointestinal tract or conjunctiva. Adenovirus types 3, 4, 7 and 14 are most commonly associated with

acute respiratory illness and types 4 and 7 have been associated with more severe outcomes than other adenovirus types, particularly in people with weakened immune systems. Adenovirus types 8, 19, 37, 53 and 54 may cause epidemic keratoconjunctivitis and adenovirus types 40 and 41 typically cause gastroenteritis, particularly in children (James, 2023).

Like noroviruses, adenoviruses are non-enveloped viruses and are stable in water (Ogorzaly et al., 2010; Rigotto et al., 2011). Human-derived contamination is the only known source of human adenovirus, and adenoviruses have commonly been used as a viral indicator of water treatment efficacy (Formiga-Cruz et al., 2005; Irving & Smith, 1981; Jiang, 2006; Pina et al., 1998; Puig et al., 1994; Rames et al., 2016). The occurrence and concentration of human adenoviruses in sewage have little seasonal variability (Jiang, 2006; Pina et al., 1998), and they are known to remain infectious in recreational and drinking water for a prolonged period of time (Ogorzaly et al., 2010; Teunis et al., 2016). These prevalence and density attributes contrast those of some other human viruses, such as enteroviruses, which demonstrate substantial seasonal variability (Rames et al., 2016). Adenoviruses can be measured by culture or molecular methods, such as PCR (Jiang et al., 2022). With a double-stranded deoxyribonucleic acid (DNA) genome, adenoviruses are more resistant to ultraviolet (UV) disinfection than other viral pathogens during wastewater treatment, as research indicates that replication of the virus after UV light exposure may be based on the cell's ability to repair damage to the DNA (Eischeid et al., 2009; Gerba & Betancourt, 2019).

The diversity of adenoviruses presents complexity in terms of exposure interpretation for their use as an enteric waterborne QMRA viral reference pathogen. In the five adenovirus challenge studies available in the scientific literature, the viral types measured (adenovirus 4/7/16) are not those that are of most concern for waterborne exposures (adenovirus 40/41), and only one included an oral exposure route. These challenge studies used culturable virus units. The currently available infection and illness dose-response models are therefore influenced primarily by inhalation exposure to adenovirus types commonly associated with respiratory infections, yielding substantial uncertainty in interpretation for water reuse applications where enteric (fecal-oral) pathogens are the primary concern (Teunis et al., 2016). Moreover, data on the health effects of ingestion exposure are inconclusive, with all subjects challenged by adenovirus 4/7 becoming infected but not producing clinical symptoms (Teunis et al., 2016). Although the inhalation dose-response may be adequate for considering a potential risk from aspiration of ingested water or exposure to aerosols (Carducci et al., 2018), Jahne et al. (2024) inferred that the underlying data are currently inadequate for deriving widely applicable and health protective LRTs for water reuse exposures to enteric adenoviruses.

In summary, norovirus and adenovirus encompass a diverse set of physical characteristics that are relevant for other known and unknown human enteric viruses. However, a better understanding of the dose-response relationship for ingested enteric adenoviruses is a critical data gap that limits its applicability for deriving health protective LRTs for water reuse applications. Culturable, treatment-resistant viruses such as enterovirus or adenovirus can nonetheless be used to ensure the log reduction values (LRVs) are achieved for broad classes of viruses of concern (Jahne et al., 2024).

Protozoan Parasites

Protozoa are microscopic, single-celled organisms that can be free-living or parasitic in nature. They may replicate in humans and other warm-blooded animals, which contributes to their survival. Transmission of parasitic protozoa that live in human or animal intestines typically occurs through a fecal-oral route, such as exposure to contaminated food or water (Centers for Disease Control and Prevention, 2024a). Currently, the pathogenic protozoa most commonly associated with wastewater in the U.S. are *Cryptosporidium* spp. and *Giardia lamblia/duodenalis/intestinalis* (referred to as *Giardia* spp.) (Collier et al., 2021). Other protozoan pathogens such as *Entamoeba* spp. are generally associated with poor sanitation conditions in developing regions yet may be of concern for marginalized communities or those with high rates of international travel (Kantor et al., 2018).

Parasitic protozoa are of concern in water reuse applications because they are often detected in sources of water used for water reuse, including untreated municipal wastewater and secondary wastewater effluent (Bitton, 2005; Rose et al., 1996). *Giardia* spp. and *Cryptosporidium* spp. are also present in the feces of farm animals and ruminants, among other animals, and thus may be present in sources of water that are not subject to human contamination (Robertson, 2013). They have a relatively high resistance to treatment and disinfection (particularly the cysts [*Giardia*] and oocysts [*Cryptosporidium*] of the protozoan life cycle) and the ability to cause infection at low doses typical of environmental exposures (Messner & Berger, 2016a; Nappier et al., 2018). Exposure to these protozoan parasites generally results in self-limiting, acute gastroenteritis in healthy adults. However, more serious and/or life-threatening outcomes, such as severe debilitating diarrhea with weight loss and malabsorption, as well as long-term sequelae, are known to occur in immunocompromised individuals (Abubakar et al., 2007).

Cryptosporidium spp.

Cryptosporidium spp. is included in the enteric waterborne QMRA reference pathogen list because it is one of the most common parasitic causes of illness in the United States, has relevant peer-reviewed dose-response relationships, may be transmitted through a waterborne route of exposure, is known to be resistant to water and wastewater treatment, and is consistently found in sources of water used for water reuse (Bitton, 2005; Collier et al., 2021; Rose et al., 1996; Rose et al., 1991a; Scallan et al., 2011; Soller et al., 2017a).

Cryptosporidium spp. is shed in feces by humans and other animals as an oocyst, which has a hard, environmentally resistant shell (Messner & Berger, 2016a). Bovine species (such as cattle, water buffalo and yaks), small ruminants (such as sheep and goats), and several other animals (such as pigs, deer, rabbits and poultry) can shed *Cryptosporidium* spp. including *C. parvum, C. bovis, C. andersoni, C. ryanae, C. meleagridis* and *C. baileyi* (Robertson, 2013). The infectivity of those *Cryptosporidium* species can vary substantially (Teunis et al., 2002b). Of the nearly 20 *Cryptosporidium* species and genotypes that have been reported in humans, *C. hominis* and *C. parvum* are responsible for the majority of documented infections (Leoni et al., 2006; Ryan et al., 2014).

Cryptosporidium spp. has been responsible for numerous waterborne outbreaks, including the 1993 outbreak in Milwaukee, Wisconsin, which is estimated to have resulted in approximately 403,000 illnesses, and a 2010 outbreak in Osterland, Switzerland, which resulted in approximately 27,000 illnesses. The CDC estimates *Cryptosporidium* illness affects approximately 825,000 U.S. residents

annually (Collier et al., 2021). *Cryptosporidium* was also a key component in the EPA Long Term 2 Enhanced Surface Water Treatment Rule (U.S. EPA, 2006c).

Giardia spp. *Giardia* spp. is included in the enteric waterborne QMRA reference pathogen list because it is the most common cause of parasitic illness in the United States, has relevant peer-reviewed dose-response relationships, may be transmitted through a waterborne route of exposure, is known to be resistant to water and wastewater treatment and is consistently found in sources of water used for water reuse (Bitton, 2005; Collier et al., 2021; Rose et al., 1996; Rose et al., 1991a; Scallan et al., 2011; Soller et al., 2017a). The CDC estimates illness from *Giardia* affects approximately 1,100,000 U.S. residents annually (Collier et al., 2021).

Giardia spp. may be present in a variety of sources that could be relevant to water reuse, including wastewater (Enriquez et al., 1995; Grimason et al., 1996; Roach et al., 1993; Sykora et al., 1991), surface waters (Chauret et al., 1995; LeChevallier et al., 1991; States et al., 1997) and numerous animal sources (Appelbee et al., 2005). *Giardia* spp. from some animals exhibit an apparent high degree of host specificity; other isolates may infect more than one host. Studies indicate that cross-species transmission of *Giardia* spp. can occur (U.S. EPA, 1999). However, most species of *Giardia* are host adapted, except for *G. duodenalis*, which seems to have a much broader host range and infects many mammalian species (Appelbee et al., 2005).

Together, *Cryptosporidium* spp. and *Giardia* spp. account for over 90% of all estimated annual gastrointestinal illnesses in the United States caused by parasitic protozoa, and thus enteric waterborne QMRA studies focused on these two protozoa are considered comprehensive and representative of the pathogen group. Some water reuse QMRAs used to develop LRTs have considered both *Cryptosporidium* spp. and *Giardia* spp. and selected the one with the more conservative LRT value (Schoen et al., 2023). Others recognize the distinction in treatment performance between the two pathogens (*e.g.*, chlorine resistance of *Cryptosporidium*) and specify LRTs for each separately. For example, the State of California specifies different LRTs for *Cryptosporidium* and *Giardia* for direct potable reuse with the understanding that they react differently to various types of water treatment (California Water Boards, 2024; Gerrity et al., 2023; Pecson et al., 2022a; Pecson et al., 2023).

Chapter 3. Reference Pathogen Densities in Water Reuse Sources

Sources of water for reuse considered in this report include untreated municipal wastewater, untreated onsite wastewater, graywater, stormwater and roof runoff (rainwater). These represent common fecal-contaminated sources in urban settings that have been included in previous risk-based state regulations (U.S. EPA, 2023). This chapter summarizes densities (*i.e.,* concentrations) of the six reference pathogens discussed in the previous chapter—enteric viruses (represented by norovirus and adenovirus), two protozoa species (*Cryptosporidium* spp. and *Giardia* spp.) and two bacterial species (*Campylobacter* spp. and *Salmonella enterica*)—in the various sources of water for water reuse QMRA. The following characterization of reference pathogen densities is intended to represent their temporal and spatial distributions in the United States. These densities derive from peer-reviewed literature, government reports and gray literature.

The available environmental data for each source of water is used to estimate pathogen density distributions and ranges. To the extent that peer-reviewed, formal meta-analyses that comprise multiple locations and time points are available, this report considers those characterizations to be the state of the science for generating broadly representative risk estimates. When geographically and temporally-based meta-analysis characterizations are not available, peer-reviewed literature data can be used to establish feasible and credible ranges of pathogen densities. These ranges are then characterized as uniform distributions or log-uniform distributions, if the parameter range spans more than two orders of magnitude. The uniform distribution approach of using peer-reviewed data to establish feasible ranges minimizes spatial and temporal biases by treating all available data equally without implicit or explicit preference or over/under weighting any specific dataset based on sample size (Eisenberg et al., 2002). The uniform distribution approach has a strong statistical basis and has been used widely within waterborne QMRA and epidemiological research (Boehm et al., 2018; Brown et al., 2017; Eisenberg et al., 2002; Schoen & Ashbolt, 2010; Soller et al., 2010a; Soller et al., 2015; Soller et al., 2017a; Viau et al., 2011).

An alternative data consolidation framework was recently reported for identifying and incorporating high-quality datasets into single, aggregated distributions that could be used for water reuse applications in cases for which sufficient representative data are available (Darby et al., 2023). Their inclusion criteria comprise characteristics about the sample matrix, geographic location, method sensitivity, correction for recovery efficiency, compatibility with dose-response models, sample size and temporal distribution. In that report, the authors combined six studies to characterize *Cryptosporidium* spp. densities, two of which were peer reviewed, and five studies to characterize *Giardia* spp. densities, two of which were peer reviewed. This framework is useful yet limited by the available primary data for generating broadly representative estimates and informing general policies applicable across different states and sites.

In some cases, sufficient site-specific data have been or may be collected to generate distributions that are representative of specific facilities or locations (*e.g.*, a large dataset characterizing California municipal wastewater (Pecson et al., 2022b). For assessments of specific facilities or locations, this approach may be preferred because of its specificity, provided that sufficient data are collected to be considered broadly representative of the facility or facilities (Lim et al., 2017). However, distributions

generated using data from a specific facility or a limited number of facilities over a specific period of time may not be geographically and temporally representative of other facilities, even if they have similar characteristics. Research is needed to better understand the distinction between data that are site or regionally specific compared to those that could be considered broadly representative.

For some onsite waters, monitoring data are limited, unavailable or have poorly understood variability (Jahne et al., 2017; Jahne et al., 2023; Kusumawardhana et al., 2021; Schoen & Garland, 2017). Schoen et al. (2017) specified minimum criteria for using direct pathogen observation data and found that onsite-generated sources of water generally lack characterization studies meeting these criteria. In such cases, pathogen density characterizations must rely on various modeling approaches that are applicable to the water of interest, according to its primary type and level of fecal contamination (*e.g.*, human feces, sewage or animal sources).

The values described in the following sections are intended as default values for generalized risk assessment.

Untreated Municipal Wastewater

For untreated municipal wastewater, the densities of enteric waterborne reference pathogens (Table 1) are based on literature reviews in peer-reviewed publications related to potable reuse QMRA studies (Nappier et al., 2018; Soller et al., 2018b; Soller et al., 2017a), supplemented with additional, more recent data from peer-reviewed publications, as available.

Table 1. State-of-the-science densities of waterborne QMRA reference pathogens in untreated municipal wastewater.

Reference Pathogen	Units	Distribution[a] (log_{10}/L)	References
Norovirus GI + GII	genome copies	N(4.7,1.5)	Eftim et al. (2017)
Adenovirus	IU	U(1.75, 5.1)	Hewitt et al. (2011); Hurst et al. (1988); Pecson et al. (2022b)
Cryptosporidium spp.	oocysts	U(-0.5,4.38)	Crockett (2007); Harwood et al. (2005); Kitajima et al. (2014); Madore et al. (1987); Nasser (2016); Pecson et al. (2022b); Robertson et al. (2006); Yang et al. (2015)
Giardia spp.	cysts	U(0.5,5.0)	Harwood et al. (2005); Kitajima et al. (2014); Pecson et al. (2022b); Sykora et al. (1991); Wallis et al. (1996)
Campylobacter spp.	CFU	U(2.95,4.6)	Stampi et al. (1993)
Salmonella enterica	CFU	U(0.5,7.4)	Bonadonna et al. (2002); Jimenez-Cisneros et al. (2001); Lemarchand and Lebaron (2003)

Note: Values shown in the table are log_{10} values.
[a] N is Normal (mean, standard deviation), U is Uniform (min, max).

Given the epidemiological importance of the topic, an increasing amount of literature has been published over the last two decades that is useful for characterizing norovirus densities in wastewater (Aw & Gin, 2010; Katayama et al., 2008; Katayama et al., 2004; Lodder & de Roda Husman, 2005; Rose et al., 2004; van den Berg et al., 2005). The concentration of norovirus in untreated municipal wastewater is now well characterized by meta-analyses that are broadly representative of the United States and the world (Eftim et al., 2017; Pouillot et al., 2015). Eftim et al. (2017) conducted a systematic literature

review of published peer-reviewed publications to identify norovirus density data in wastewater influent globally and developed a meta-analysis approach for developing viral pathogen density distributions. Their analysis includes approximately 850 data points—accounting for heterogeneity in study-specific distribution curves, sampling locations and sampling season—to provide a comprehensive and updated representation of the data. Results indicate a high density of norovirus in wastewater influent (overall mean approximately $10^{4.8}$ GC/L with values as high at $10^{9.2}$ GC/L), with a higher density of norovirus genogroup GII (mean $10^{4.9}$ GC/L) than GI (mean approximately $10^{4.6}$ GC/L). The analysis also accounts for differences in seasonal and geographical occurrences of norovirus GI and GII. The norovirus parameter values (normal distribution with mean $10^{4.7}$ GC/L and standard deviation of $10^{1.5}$ GC/L) represent the analysis results for all data by pooling the norovirus genogroups and accounting for seasonality (Table 1).

More recently, Pecson et al. (2022b) reported the results from approximately 120 observations of norovirus in untreated municipal wastewater from five wastewater treatment plants in California during a 14-month monitoring campaign designed to support statewide regulations for DPR. Their mean norovirus results were slightly lower (approximately $10^{3.8}$ GC/L) than those reported by Eftim et al. (2017). However, those data were largely collected during COVID-19 pandemic disruptions and were "significantly lower than literature values of norovirus densities in non-pandemic years" (Wigginton et al., 2021). In addition, public health data from the CDC confirm that norovirus outbreaks were significantly lower in 2020 than in other years, with only two outbreaks reported from March 2020 to December 2020 in the entire United States. By comparison, there were 86 norovirus outbreaks reported in January 2020 alone (Wigginton et al., 2021).

Several caveats exist for other reference pathogens in untreated municipal wastewater. For adenovirus, the lower bound of the uniform distribution is based on detectable values and thus may overestimate adenovirus densities at the lower end of the distribution, as not all reported adenovirus densities are above detectable limits. Additionally, while Table 1 summarizes adenovirus densities measured using infectious units (IU), gene copy concentrations greater than 10^8 gc/L have been observed using PCR methods (Rames et al., 2016). For *Giardia* spp. (Sedmak et al., 2005), using a uniform distribution results in a generally lower set of percentile values compared with the normal distribution (mean 4.0 $\log_{10}$ units and standard deviation 0.4 $\log_{10}$ units) derived for California facilities (WateReuse Research Foundation, 2021). For *Salmonella enterica*, densities were reported using various units including MPN and CFUs; for pathogen characterization purposes, these units were assumed to be equivalent.

Onsite Waters: Graywater, Stormwater, Untreated Onsite Wastewater and Roof Runoff

Pathogen densities for graywater, stormwater, untreated onsite wastewater and roof runoff were recently compiled and updated by Schoen et al. (2023) for the purpose of deriving pathogen treatment targets for onsite non-potable water systems. Graywater is defined as wastewater from bathtubs, showers, bathroom sinks and clothes washing machines, excluding toilet and (in most cases)dishwasher and kitchen sink wastewaters; roof runoff is defined as precipitation collected from roof surfaces or other above-ground collection surfaces not affected by human activity; and stormwater is defined as precipitation and runoff collected at ground level (Schoen et al., 2017). In that work, data were included

when the following criteria were met: 1) the analytical methods used to enumerate the pathogens were comparable with those used in the dose-response studies (*i.e.*, "conventional" methods for all reference pathogens except norovirus); and 2) if a large fraction of the samples were non-detects, the limit of detection was specified (Schoen & Garland, 2017). Sufficient direct pathogen observations are unavailable for robust characterization of density distributions in any of the onsite sources of water, necessitating modeling approaches. A summary of these pathogen densities is included below (Table 2). Of note, pathogen densities are reported for fresh collections of water only; storage may alter these concentrations through pathogen decay, growth, or subsequent contamination of stored waters (Crabtree et al., 1996; Rose et al., 1991b).

Table 2. State-of-the-science densities of waterborne QMRA reference pathogens in onsite collected waters.

Source of Water	Reference Pathogen	Units	Occurrence[c] (%)	Distribution[d] (log_{10}/L)	References
Untreated onsite wastewater	Norovirus GI + GII	genome copies	99.7	E(6.52,8.21)	Jahne et al. (2017)
	Cryptosporidium spp.	oocysts	11.3	E(3.28,5.11)	Jahne et al. (2017)
	Giardia spp.	cysts	69.8	E(2.92,4.65)	Jahne et al. (2017)
	Campylobacter spp.	CFU	27.3	E(2.75,5.35)	Jahne et al. (2017)
	Salmonella enterica	CFU	23.2	E(3.52,5.28)	Jahne et al. (2017)
Graywater	Norovirus GI + GII	genome copies	99.7	E(3.86,5.73)	Jahne et al. (2017)
	Cryptosporidium spp.	oocysts	11.3	E(0.99,2.77)	Jahne et al. (2017)
	Giardia spp.	cysts	69.8	E(0.84,2.48)	Jahne et al. (2017)
	Campylobacter spp.	CFU	27.3	E(1.13,3.19)	Jahne et al. (2017)
	Salmonella enterica	CFU	23.2	E(1.11,2.89)	Jahne et al. (2017)
Stormwater[a]	Norovirus GI + GII	genome copies	100	N(3.7,1.5)	Jahne et al. (2023)
	Cryptosporidium spp.	oocysts	100	U(-1.5, 3.38)	Schoen et al. (2023)
	Giardia spp.	cysts	100	U(-0.5, 4.0)	Schoen et al. (2023)
	Campylobacter spp.	CFU	100	U(1.95,3.6)	Schoen et al. (2023)
	Salmonella enterica	CFU	100	U(-0.5, 6.4)	Schoen et al. (2023)
Roof runoff[b]	*Campylobacter* spp.	CFU	100	E(-0.85,1.67)	Schoen et al. (2017)
	Salmonella enterica	CFU	100	E(0.15,3.94)	Schoen et al. (2017)
	Giardia spp.	cysts	100	U(-0.7,1.2)	Alja'fari et al. (2022)

[a] Based on municipal wastewater at 10% by volume.
[b] Roof runoff densities are subject to greater uncertainty than others; refer to Schoen et al. (2017; 2023) for discussion.
[c] Occurrence is modeled and is therefore not sensitive to measurement detection limits.
[d] N is Normal (mean, standard deviation), U is Uniform (min, max), E is Empirical (median, 95th percentile when occurring).

Graywater and Untreated Onsite Wastewater Densities. Pathogen monitoring data for untreated onsite wastewater and graywater from onsite reuse systems have been extremely limited (Jahne et al., 2017; Jahne et al., 2023; Kusumawardhana et al., 2021; Schoen & Garland, 2017). Therefore, the pathogen density characterizations reported by Schoen et al. (2023) use the epidemiology-based models of Jahne et al. (2017). The epidemiology-based models simulate pathogen densities in small-scale collections where their occurrence is anticipated to be sporadic relative to larger populations with more equalized input (*e.g.*, municipal wastewater) (Schoen et al., 2023). This approach uses fecal contamination levels of source-diverted graywater and mixed onsite wastewater coupled with modeled infections in relevant population sizes and the pathogen shedding dynamics of those infected individuals to simulate pathogen occurrence and density estimates in resulting water collections (Jahne et al., 2017). Of modeled population sizes (5-, 100-, and 1,000-persons), the 1,000-person simulations are used in reported estimates given the common implementation of onsite reuse in large buildings; the resulting LRTs are also protective of smaller systems (Jahne et al., 2023; Schoen et al., 2017).

Other onsite pathogen densities have been adopted as inputs for estimating onsite LRTs. Sylvestre et al. (2024) used raw shower and graywater monitoring data to characterize onsite *E. coli* concentrations rather than those reported by Jahne et al. (2017) based on summary statistics across multiple studies. When used to simulate pathogen densities in onsite waters as input to the epidemiology-based Monte Carlo approach, the resulting LRTs for combined graywater were similar using either method (Reynaert et al., 2024). In contrast to the epidemiology-based, simulated onsite pathogen concentrations, Pecson et al. (2022a) relied on the California-specific municipal wastewater dataset reported by Pecson et al. (2022b) to estimate pathogen densities in onsite wastewater (assumed equivalent to municipal) and graywater (assumed two $\log_{10}$ dilution of municipal wastewater). A comparison of the pathogen characterization approaches and their results is provided in Jahne et al. (2023). The greatest difference in pathogen densities between the approaches was for norovirus densities in graywater, where densities estimated using diluted municipal wastewater are roughly 3-$\log_{10}$ less than those simulated using the epidemiology approach of Jahne et al. (2017). The pathogen occurrence also differed between approaches. Simulated *Cryptosporidium* spp. and *Giardia* spp. pathogen densities occurred sporadically depending on infection incidence in the modeled population, while the densities estimated using municipal wastewater were assumed to occur 100% of the time.

Stormwater. Urban stormwater is inherently variable both spatially and temporally due to the diversity of sources and complexity of influencing factors in these settings (Geldreich, 1978)). To estimate generalized densities of reference pathogens in stormwater, researchers use municipal wastewater pathogen densities and assume various levels of dilution into stormwater (*e.g.*, 1:10, 1:1,000 or 1:10,000 volumetric contributions of wastewater to stormwater) based on their relative loadings of human-specific fecal markers and the predicted range of risk estimates from limited available stormwater data (Jahne et al., 2023; Pecson et al., 2022a; Schoen et al., 2017; Schoen et al., 2023). Stormwater estimates (Table 2) are derived from a recent Water Research Foundation publication (Sharvelle et al., 2023). This analysis confirmed the high variability of stormwater microbial quality, with no quantitative variables adequately predicting the levels of human fecal contamination in stormwater. The authors therefore recommended that to achieve conservative treatment a 1:10 ratio (10% sewage content) be used as the default assumption when deriving LRTs for urban stormwater collections (Sharvelle et al., 2023). This approach was also recommended for the development of onsite non-potable LRTs in California (National Water Research Institute, 2021). The values shown in Table 2 characterize densities of the relevant reference pathogens in municipal wastewater that have been reduced accordingly. However, Sharvelle et al. (2023) note that site-specific characterization of wastewater contamination in stormwater collections (*e.g.*, using host-specific human fecal markers) may be used to select an alternative dilution (*i.e.*, 1:1,000 or 1:10,000) when a site-specific monitoring program demonstrates that the system influent remains within the selected dilution.

Roof Runoff/Collected Rainwater. U.S. rainwater data suitable for waterborne QMRA are extremely limited and international studies are confounded by their different animal sources and environmental characteristics. The distribution of pathogen densities in roof runoff (rainwater) are therefore based on a modeling approach using the amount of fecal contamination in roof runoff coupled with estimated levels of pathogens in animal fecal sources (Schoen et al., 2017). However, the data for applying this model are limited to bacterial pathogens and specific fecal sources (*i.e.*, gulls) (Schoen et al., 2017).

Empirical molecular data on zoonotic pathogens are also available from U.S. roof runoff measurement studies (Alja'fari et al., 2022; Hamilton et al., 2018). Although these did not meet the required criteria of Schoen et al. (2017), the Alja'fari et al. (2022) measurements are notable for their inclusion of protozoan targets (*Cryptosporidium* spp. and *Giardia* spp. genes), which were rarely detected yet unable to be modeled using the previous approach. The values shown in Table 2 reflect those protozoan detections, despite their use of DNA targets, to allow for inclusion of protozoan LRTs which were identified as a limitation of prior analyses (Sharvelle et al., 2017). It should be noted, however, that both empirical measurements and modeling approaches remain data-limited and therefore uncertain. Data to develop roof runoff LRTs for *Cryptosporidium* spp. using either method remain unavailable.

Chapter 4. Ingestion Estimates for Potable and Non-Potable Water Exposures

The purpose of exposure assessment is to characterize the number or distribution of organisms that correspond to a single exposure (dose) or the set of exposures for a particular activity of interest (*e.g.*, daily drinking, toilet flushing) (Haas et al., 1999). Exposure is a function of magnitude (*i.e.*, number of pathogens), duration (*i.e.*, the time period over which risks are compounded) and frequency (*i.e.*, how often the exposure occurs).

For water reuse risk assessments that focus on enteric pathogens, exposures that occur on one day are considered independent of exposures that occur on other days. For daily exposures, dose magnitude is computed as the product of reference pathogen densities and the volume of water ingested during the exposure scenario for that day. It is possible that multiple exposures that occur within a single day could also be independent; however, experimental data are lacking in this regard (Pujol et al., 2009). Thus, multiple exposures that occur within a single day are considered a single combined exposure.

This chapter summarizes the best available estimates on the volume of water ingested through a range of potable and non-potable water uses, along with assumptions about exposure frequency (*i.e.*, how often exposure occurs). A duration of one year is considered for comparison to annual benchmark metrics, and thus daily risks are aggregated into annual risks based on the frequency of exposure (as described in Chapter 6).

Table 3 summarizes ingestion estimates for potable reuse (drinking water), unrestricted irrigation and common indoor non-potable uses, and also explains key assumptions underlying the estimates. Note that non-potable exposures may still result in inadvertent ingestion due to the generation of ingestible droplets and hand-to-mouth contact with the treated water. While other exposures (*e.g.*, inhalation, dermal) may also occur, risk assessments to define water reuse treatment focus on the enteric pathogens and hence on the corresponding ingestion pathway through which those pathogens initiate infection.

Table 3. State-of-the-science ingestion estimates.

End Use	Daily Exposure Volume (L)	Exposure Frequency (days/year)	Fraction of Population Exposed	Key Assumptions and Citations
Potable use	2.4	365	1	90th percentile per capita U.S. ingestion (U.S. EPA, 2011, 2019, 2024)[a]
Indoor non-potable water use	0.00004	365	1	Comprises toilet flushing and clothes washing. Adopted from Australian guidelines (National Resource Management Ministerial Council et al., 2006)
Unrestricted irrigation and dust suppression	0.001	50	1	Adopted from Australian guidelines (National Resource Management Ministerial Council et al., 2006)
Cross-connection of treated water with potable water or accidental ingestion	2	1	0.1	Added to indoor use calculation (Schoen et al., 2017; Schoen et al., 2018)

[a] U.S. EPA (2024) utilizes the same source data and percentile but selects subpopulations corresponding to the critical effect.

Drinking Water

For drinking water, the ingestion estimates derive from the EPA *Exposure Factors Handbook* (February 2019 update, Chapter 3) (U.S. EPA, 2019), which presents exposure values for individuals who consumed water during the evaluation studies (National Health and Nutrition Examination Survey [NHANES] 2005–2010). These values are the basis for the EPA's drinking water risk assessments, including the recent Proposed Per- and Polyfluoroalkyl Substances (PFAS) National Primary Drinking Water Regulation (U.S. EPA, 2024).

"Consumer-only" ingestion rates represent the quantity of water consumed only by individuals who reported water intake during the survey period (excludes individuals who did not ingest water from the source of interest) (U.S. EPA, 2019). "Per capita" ingestion rates represent intake that has been averaged over the entire population and are generated by averaging consumer-only intakes over the entire population (including those individuals that reported no intake). Therefore, rates of consumer-only ingestion of community water (*i.e.*, not including bottled water) represent drinking water ingestion estimates for the population who may consume potable reuse water. Although the *Exposure Factors Handbook* provides multiple percentile values describing this distribution, use of the 90th percentile point estimate value (2.413 liters per day, or L/d) is consistent with the risk assessment methodology for recent drinking water regulations (U.S. EPA, 2024) (Table 3). Use of an upper percentile point estimate value in risk assessment calculations is a common approach to ensure that sensitive, vulnerable and/or highly exposed sub-populations (*e.g.*, pregnant or lactating women) are protected.

Prior to publication of the 2019 update to the *Exposure Factors Handbook*, the most commonly used estimates of water ingestion were determined using the Continuing Survey of Food Intakes by Individuals (CSFII) 1994–1996 and 1998 data as reported in previous versions of the *Exposure Factors Handbook* (U.S. EPA, 2004b, 2011). The estimated 90th percentile from the distribution of daily average per capita community water ingestion was 2.014 L/d, and the 95th percentile was 2.544 L/d. The reported 90th percentile value, the 95th percentile value and the corresponding water ingestion distribution have been commonly used in previous chemical risk assessments and QMRAs related to drinking water and potable reuse (Amoueyan et al., 2017; Asano et al., 1992; Chaudhry et al., 2017; Soller et al., 2018b; Soller et al., 2017a; U.S. EPA, 2006c, 2018).

Unrestricted Irrigation

For ingestion during unrestricted irrigation (*i.e.*, ornamental plant or non-food irrigation and dust suppression with unrestricted public access), exposure estimates derive from the *Australian Guidelines for Water Recycling: Managing Health and Environmental Risks* (Australian Guidelines) (National Resource Management Ministerial Council et al., 2006). The Australian Guidelines suggest that the values presented there could be used as default values where specific or local data are not available and that the values are thought to be conservative (health protective) based on the underlying assumptions used to develop those values.

For unrestricted irrigation, both Schoen et al. (2023) and Pecson et al. (2022a) used the Australian Guidelines values for municipal irrigation and assumed that 1×10^{-3} L was ingested 50 times per year. The frequency was selected for a dry climate (*e.g.*, San Francisco, California) and may not be applicable to a climate with fewer irrigation needs. The ingestion volume is assumed to reflect indirect ingestion via

contact with lawns (National Resource Management Ministerial Council et al., 2006). Schoen et al. (2017) estimate that the ingested volume of 1×10^{-3} L is equivalent to 10–100 seconds of hand-to-mouth exposure (de Man et al., 2014c) or one drop of water (de Man et al., 2014a). Schoen et al. (2017) also explored the impact of alternative ingestion values through numerical sensitivity analyses and determined that an order of magnitude increase in ingestion volume or the number of annual exposures results in an LRT increase of approximately one.

Indoor Use

The primary indoor uses of recycled water for which LRTs have been developed include toilet flushing and clothes washing (Jahne et al., 2023; Sharvelle et al., 2017). During and following these uses, ingestion exposure may occur via generated droplets or hand contact with bathroom and laundry surfaces, both routine and during cleaning or repair activities. For these types of exposures, the ingestion volume estimates are adopted from the Australian Guidelines (similar to unrestricted irrigation). Additional ingestion exposure may result from accidental ingestion of the non-potable water (*e.g.*, by children) or from potential cross-connections of dual reticulation systems with drinking water mains. Schoen et al. (2017) and subsequent QMRAs (Pecson et al., 2022a; Schoen et al., 2023) have considered indoor uses to include toilet flush water (3 times per day), clothes washing (once per day), and rare accidental cross-connections with drinking water or direct ingestion of treated non-potable water (a one-day potable water exposure affecting 10% of the user population each year).

Analyses by Lim et al. (Lim et al., 2015) suggest that the volume of water inhaled after toilet flushing is low, approximately 10^{-9} L per event. For water ingested during hand-to-mouth exposures, Schoen et al. (2017) used children's exposure data reported by de Man et al. (2014a) to estimate that one second of wet hand-to-mouth contact could result in ingested volumes of 2×10^{-5} to 3×10^{-4} L. Both estimates support the Australian Guidelines of 3×10^{-5} L/d for toilet flushing (3 flushes $\times 10^{-5}$ L per flush) and 1×10^{-5} L/d for clothes washing with recycled water as health protective, conservatively assuming 100% partitioning and/or recovery for aerosol or hand-to-mouth exposures (National Resource Management Ministerial Council et al., 2006; Schoen et al., 2017).

For estimating potential cross-connections of dual reticulation systems with drinking water mains, few empirical data exist to characterize or estimate the rates of these events. Hambly et al. (2012) reported that "despite the significant presence of dual reticulation systems across the USA, very limited research into cross-connection events and cross-connection detection has been published," and a literature review conducted by Schoen et al. (2018) for cross-connection characteristics returned zero results for onsite non-potable water system cross-connection events in the United States. Given the lack of definitive data, Schoen et al. (2017) assumed that one accidental ingestion event or cross-connection of treated water with potable water (2 L/person/day) affects 10% of the user population one day per year, an assumption that was subsequently adopted in later analyses (Pecson et al., 2022a; Schoen et al., 2023). To assess this assumption, Schoen et al. (2018) explored scenarios for cross-connection conditions (*i.e.*, different system sizes, event durations, fractions of users exposed and intrusion dilutions) that would exceed predetermined health benchmarks. Their results indicate that the assumed cross-connection exposure factor included in the 2017 indoor use LRTs (*i.e.*, one day of potable ingestion by 10% of the population) is protective for isolated, short-duration reclaimed-to-potable events (*e.g.*,

0.1% of user population affected for less than 5 days). Additionally, in examining the impact that the cross-connection exposure had on calculated LRTs, Schoen et al. (2018) determined that the calculated LRTs would decrease by one log or less if this protection factor were omitted (*i.e.*, for indoor use comprising toilet flushing and clothes washing only).

Other Non-Potable Uses

Other potential non-potable applications of recycled water include fire suppression, car washing and decorative fountains (Arden et al., 2020; Jahne et al., 2023; Pecson et al., 2022a; Schoen et al., 2017; Schoen et al., 2020b). Empirical data are lacking to inform the exposure assumptions related to ingestion volumes and exposure frequency for most of these other, less commonly practiced non-potable uses (Jahne et al., 2023). Therefore, Schoen et al. (2020a) assessed the sensitivity of indoor use LRTs to potential exposure volumes from other uses. Their results indicate that the indoor use LRTs may also be adopted for other non-potable applications with anticipated ingestion volumes of roughly 10^{-5} L or less, provided that the cross-connection factor is included (*i.e.*, potable exposure for 10% of population one day per year). For less frequent uses, this ingestion volume may be assumed to increase to less than roughly 10^{-4} L for exposures of 50 days per year or fewer. The authors estimate that these uses include reuse in decorative fountains and vehicle washing, each with an approximate routine exposure of 10^{-4} L based on water features in splash parks (de Man et al., 2014a; de Man et al., 2014b) and high-pressure vehicle washing (Sinclair et al., 2016), respectively.

In contrast, Pecson et al. (2022a) estimated specific exposure volumes for these end uses to model respective LRTs. For car washing, Pecson et al. (2022a) assumed an ingestion volume of 0.001 L at a frequency of 12 days per year, based on the assumption that ingestion during car washing would be similar to indirect ingestion during garden irrigation as estimated by the Australian Guidelines (National Resource Management Ministerial Council et al., 2006). An underlying difference in the car washing estimates by Schoen et al. (2020a) and Pecson et al. (2022a) is that the former is based on use of a spray device whereas the latter is assumed for washing by hand. For indoor decorative fountains, Pecson et al. (2022a) assumed that ingestion volumes would be similar to those for high-pressure car washing as reported by Sinclair et al. (2016), but presented two statistical distributions (normal and uniform) fitted to the same data. These were considered in separate “indoor use” combinations, in addition to the toilet flushing, clothes washing and cross-connection exposures above. Pecson et al. (2022a) also included fire suppression, for which they assumed an ingestion volume of 0.002 L at a frequency of 20 days per year based on assumptions from the Water Services Association of Australia (Deere et al., 2004).

There is still uncertainty associated with these additional end uses and a lack of consensus among different studies. However, Schoen et al. (2020a) and Pecson et al. (2022a) both concluded that indoor use LRTs are also protective for decorative fountains and vehicle washing (as well as fire suppression in Pecson et al. (2022a)). Pecson et al. (2022a) also calculated that LRTs for unrestricted irrigation were protective for fire suppression and car washing, but not for decorative fountains.

Chapter 5. Dose-Response Models for Reference Pathogens

The objective of microbial dose-response assessments is to develop a mathematical relationship between the level of microbial exposure and the likelihood of an adverse health effect (Haas et al., 1999). A dose-response model is a mathematical function that uses the estimated pathogen dose (Chapter 4) to yield the probability of a selected adverse health effect, such as the probability of infection or the probability of illness for exposed individuals.

When pathogenic organisms enter the human body, they encounter a system of barriers to infection. Enteric pathogens must successfully reach parts of the intestinal tract that are suitable for attachment, colonization and/or replication (Teunis et al., 1999). For infection to occur, at least one of the ingested pathogens must survive to start colonization or replication. This biological process forms the underlying mathematical basis for dose-response models used for QMRA (Haas et al., 1999; Teunis et al., 1999).

Detailed mathematical derivations of commonly used dose-response models for infection have been published previously (refer to Haas et al. (1999, 2014), Teunis et al. (1999) and Mitchell and Weir (2024)). However, not all infections result in symptomatic illnesses, and the likelihood of this transition is described by a conditional probability. The conditional probability of illness given infection takes two forms: dose-independent and dose-dependent (*e.g.*, increasing illness risk for increasing dose). The recent dose-dependent illness characterizations for norovirus and *Campylobacter* spp. incorporate challenge and outbreak data within a hierarchical model to develop the correspondence between dose and illness, whereas the dose-independent characterizations (*e.g.*, *Salmonella enterica* and *Giardia* spp.) assume that illness is not correlated to initial dose.

The most current waterborne QMRA dose-response models for probability of infection and conditional probability of illness given infection, along with the corresponding parameter values and citations, are summarized in Table 4 for key enteric reference pathogens (Chapter 2). With the constraint of limited human or animal challenge and outbreak data to inform dose-response relationships, the strains used to develop the models in Table 4 represent each class of reference pathogen, although they do not necessarily capture the full variability of all species and/or strains.

Table 4. Dose-response models and parameter values for infection and illness for waterborne pathogens; adapted from Schoen et al. (2023).

Pathogen	Units	Infection Model	Parameter	Value	Illness Model[d]	Parameter	Value	References
Norovirus GI/GII SE+[a]	Genome copies	Hyper-geometric	α β	0.393 0.767	Dose-dependent function with bivariate normal parameters	Mean (w) Mean (z) Variance (w) Covariance (w,z) Variance (z)	1.74 1.82 5.55 −0.708 4.64	Teunis et al. (2020)
Adenovirus	IU	Hyper-geometric	α β	5.11 2.8	Dose-dependent function	η r	6.53 0.41	Teunis et al. (2016)
Cryptosporidium spp.[b]	Oocysts	Fractional Poisson	P	0.737	Dose-independent uniform	Minimum Maximum	0.3 0.7	Messner and Berger (2016a) U.S. EPA (2006c)
Giardia spp.	Cysts	Exponential	r	0.0199	Dose-independent uniform	Minimum Maximum	0.2 0.7	Rose et al. (1991a) Eisenberg et al. (1996)
Campylobacter spp.[c]	CFU	Hyper-geometric	α β	0.44 0.51	Dose-dependent parameters function with bivariate normal parameters	Mean (w) Mean (z) Variance (w) Covariance (w,z) Variance (z)	−2.744 −0.0049 1.337 0.01 0.993	Teunis et al. (2018)
Salmonella spp.	CFU	Beta-Poisson	α β	0.3126 2884	Dose-independent uniform	Minimum Maximum	0.17 0.4	Fazil (1996) Haas et al. (1999)

[a] The values shown are the median GI predicted parameter values for infection and transformed dose response parameters for illness, the latter based on shellfish outbreak data.
[b] The fractional Poisson, exponential with immunity and hyper-geometric fit the available data equivalently well; refer to Messner and Berger (2016a).
[c] The values shown are the median *C. jejuni* predicted parameter values for infection and transformed dose-response parameters for illness, the latter based on challenge study data.
[d] The illness model is conditional probability of illness given infection.

Bacterial Reference Pathogens

Campylobacter spp.

The dose-response relationship for the reference pathogen *Campylobacter* spp. is assumed to follow that *of C. jejuni*, the species most associated with human infection (Centers for Disease Control and Prevention, 2024b). The *C. jejuni* infection dose-response relationship (Table 4) incorporates both challenge and outbreak data from different host species (primates and humans) and different strains of *C. jejuni* (Teunis et al., 2018). All challenge studies involved high pathogen doses and a dose-response effect (increasing infection trend with dose) was observed in only one study. However, using a hierarchical framework, the additional outbreak data provided valuable information on infection in the low dose range. A high susceptibility to infection with *C. jejuni* was found in a combined analysis of challenge studies and outbreaks, four of which were associated with raw milk consumption. The generalized infection dose-response relationship for *C. jejuni* uses these multiple sets of data and captures the variation in infectivity across *C. jejuni* strains (Teunis et al., 2018). The hyper-geometric parameter values for infection are the reported median parameter estimates (Table 4).

While the outbreak and challenge data were used jointly to generate a generalized infection dose-response relationship, there were substantial differences between the outbreak and challenge illness

study results (Teunis et al., 2018). The parameter values for illness (Table 4) are based on challenge data given the high selection bias of the population for the outbreak studies (mainly young children) (Schoen et al., 2023). The illness parameter values are inputs to a dose-dependent illness function (Teunis et al., 2018). A bivariate normal function is used to capture the uncertainty in the illness parameter values by creating paired samples of the illness parameters in a Monte Carlo analysis (Teunis et al., 2018). The use of the median values for infection parameter values (*i.e.*, hyper-geometric parameters α and β in Table 4) with the bivariate normal distribution for the illness parameter values results in a significant reduction of computational complexity with minimal effect on calculated risk estimates (Schoen et al., 2023).

Previously, the most widely used dose-response relationships for *C. jejuni* were those reported by Medema et al. (1996) and Teunis et al. (2005). Medema et al. (1996) reported a beta-Poisson model fitted to experimental data of *C. jejuni* infection obtained in human feeding studies performed by Black et al. (1988). The occurrence of symptoms of intestinal illness did not follow a dose-response trend and an absence of experimental data in the low dose range resulted in relatively large confidence intervals at low doses. Subsequently, Teunis et al. (2005) evaluated an incident at a dairy farm where several children became ill as a result of drinking raw milk contaminated with *C. jejuni*. These data appeared to show a nearly exponential dose-response relationship between the amount of milk consumed and the illness attack rate, conflicting with the rather slowly rising dose-response relationship established in the previously reported feeding study. Teunis et al. (2005) reconciled this by considering illness and infection separately, which was the approach used in the dose-response model in Table 4 (Teunis et al., 2018).

Salmonella spp.

For the reference pathogen *Salmonella* spp., the dose-response relationship for infection is based on work conducted by Fazil (1996) and published by Haas et al. (1999). Dose-response data were obtained from human challenge studies conducted by McCullough and Eisele (McCullough & Eisele, 1951a, 1951b) on six nontyphoid strains. The beta-Poisson model provided a statistically significant fit to the data once several outlier data points were removed. It can, however, be noted that outbreaks of salmonellosis frequently involve strains that were not included in the original challenge studies (*i.e.*, *S. typhimurium* and *S. enteritidis*), so there is uncertainty about the applicability of the model to these strains (Haas et al., 1999).

The dose-response parameter values for illness (Table 4) represent the data summarized by Teunis et al. (1999) for a challenge study on *Salmonella enterica* serovar *meleagridis* (Strain III) in human subjects (McCullough & Eisele, 1951a). Since limited data were available to characterize the conditional probability of illness given infection, the minimum and maximum observed fractions of ill individuals are used as bounds of a uniform distribution. Dose-response parameter values for illness are available for other serovars associated with foodborne outbreaks in various food matrices (Teunis et al., 2010).

Viral Reference Pathogens

Norovirus

For the reference pathogen norovirus, the current infection dose-response relationship incorporates both challenge and outbreak data based on norovirus genome copies (Teunis et al., 2020), which is the same methodology used for measuring reference pathogen data in the sources of water evaluated for water reuse. This study additionally considers host factors such as infectivity by secretor status (Johnson et al., 2022), and the pathogenicity of different genogroups. Data included the results from 14 challenge studies using six different norovirus isolates (G1.1 [8fIIa], G1.1 [8fIIb], GI.1 [42399], GII.2 [SMV], GII.4 [Farmington Hills], GII.1 [Hawaii]) and data from seven oyster-related norovirus outbreaks that caused gastrointestinal illness. Five of those outbreaks had been used in a previously published outbreak-based dose-response assessment (Thebault et al., 2013). Although data from any single outbreak may add only incremental information, they can be combined to develop a dose-response relationship (Thebault et al., 2013). The outbreak dose-response assessment by Thebault et al. (2013) showed infection results consistent with previous challenge study outcomes by Teunis et al. (2008). Teunis et al. (2020) also noted that "the reports claiming low infectivity of norovirus ignore the outbreak-based estimates that were available (Thebault et al., 2013). Although many would consider outbreaks a highly uncertain source of information, the importance of outbreak data in establishing infectivity should not be underrated." The Teunis et al. (2020) meta-analysis results combining both outbreak and feeding study data confirm the high infectivity of norovirus in these settings, with an estimated mean infection risk of 28% when exposed to 1 GC of norovirus GI, and 7.6% for 1 GC of norovirus GII, both in secretor-positive subjects.

The median dose-response parameter values shown in Table 4 for infection represent the most infectious GI genogroup, as suggested for exposures to unknown strains by Teunis et al. (2020), and the serogroup positive population (Se+) dose-response characterization (*i.e.*, the most susceptible group) to account for susceptibility to the wide range of norovirus variants and norovirus-like pathogens that are potentially present in an untreated source, following Schoen et al. (2023).

In contrast to the infection dose response in which the outbreak and challenge data resulted in similar parameter values, the illness dose-response parameter values for outbreak indicated higher illness risk than the feeding study data (Teunis et al., 2020). The dose-response parameter values for illness (Table 4) are based on shellfish outbreak data and are also for the more conservative GI Se+ characterization, given the wide range of strains captured in these outbreaks, despite the uncontrolled characteristics of the exposed population which may include immune-compromised subjects (Schoen et al., 2023). The illness parameter values are inputs to a dose-dependent illness function (Teunis et al., 2018). A bivariate normal function is used to capture the uncertainty in the illness parameter values by creating paired samples of the illness parameters (Teunis et al., 2018).

The initial norovirus dose-response relationship was published by Teunis et al. (2008), yielding high estimates of infectivity for exposure to a single genome copy of the prototypical Norwalk virus (genotype GI.1). Another challenge study with the same virus followed (Atmar et al., 2014) and, given the lack of dose-response studies for other noroviruses, this dose-response relationship was used as a model for norovirus generally (Teunis et al., 2020). Since the initial Teunis et al. (2008) publication, data

from the same Norwalk virus challenge studies have been re-evaluated with alternative mathematical models that generate different outcomes (Messner et al., 2014; Schmidt, 2015), creating uncertainty within the risk assessment community regarding a single "consensus" model for recommended use in risk assessment (Chaudhry et al., 2017; Lim et al., 2017; Olivieri et al., 2014; Pecson et al., 2022a; Schoen et al., 2011; Soller et al., 2010a; Soller et al., 2010b; Viau et al., 2011; Water Research Foundation, 2021). To address this concern, Van Abel et al. (2017) recommended the use of multiple approaches, which was implemented to develop water reuse LRTs (Pecson et al., 2022a; Schoen et al., 2017). Likewise, Soller et al. (2017b) developed an approach that accounted for uncertainty within the norovirus dose-response realm by randomly weighting the different models. Teunis et al. (2020) addresses the debate about infectivity by incorporating additional challenge study and outbreak data to develop a more representative, generalized model that is less sensitive to concerns about any one dose-response data set (including the initial inoculum with unknown aggregation status) (Schoen et al., 2023). The updated norovirus dose-response is used in recent water reuse risk assessments (Jahne et al., 2024; Reynaert et al., 2024; Schoen et al., 2023) and other QMRA studies (Ahmed et al., 2024; Li et al., 2023; Skiendzielewski et al., 2024).

Adenovirus

For the reference pathogen adenovirus, the current dose-response relationships describe the distributions of infectivity and pathogenicity from data reported in five challenge studies of adenovirus subgenus E (adenovirus 4) and subgenus B (adenovirus 7 and 16) (Teunis et al., 2016). These adenovirus types (adenovirus 4/7/16) are commonly associated with respiratory infections and the challenge studies did not include data for the adenoviruses that cause gastrointestinal illness and are frequently measured in wastewater (adenovirus 40/41) (Couch et al., 1966; Couch et al., 1963; Couch et al., 1969; Hitchcock et al., 1960; Kasel et al., 1963). The Teunis et al. (2016) study developed dose-response models using a hierarchical framework for four different modes of inoculation used in the dose-response studies: oral ingestion, inhalation, intranasal and intraocular droplet inoculation. The hierarchical framework assumes that all virus types and inoculation routes share a common dose-response shape that shifts for each route. However, the oral ingestion data provided minimal information for the dose-response relationship in comparison to the inhalation route, resulting in an oral dose-response model that has substantial uncertainty (wide 95% predictive interval) and very similar parameter estimates to the inhalation route that comprises the majority of available data (Teunis et al., 2016). Conclusions regarding oral exposure were further complicated by the observation that all exposed subjects became infected without producing clinical symptoms.

The previously used dose-response relationship, often attributed to Crabtree et al. (1997), likewise relied on inhalation data of adenovirus 4. This lack of data specific to oral ingestion yields substantial uncertainty in the interpretation of adenovirus risk for water reuse applications where enteric pathogen (fecal-oral adenovirus 40/41) exposure via waterborne transmission is the primary concern. Because of this, adenovirus dose-response relationships are considered inadequate for deriving widely applicable and health protective LRTs for water reuse exposures to enteric pathogens (Jahne et al., 2024), and are not included in Chapter 7 of this report. The dose-response relationship for enteric adenovirus is an outstanding research need.

Parasitic Protozoan Reference Pathogens

Cryptosporidium spp.

For the reference pathogen *Cryptosporidium* spp., the most updated infection dose-response relationship is based on work published by Messner and Berger (Messner & Berger, 2016a). Messner and Berger (2016a) used human dose-response data from seven *Cryptosporidium* isolates (species *C. parvum*, *C. hominis* and *C. muris*) to investigate six models of varying complexity that estimate infection probability as a function of dose. Previous models (summarized below) attempted to explicitly account for virulence differences among *C. parvum* isolates. Three models—the fractional Poisson, exponential with immunity, and hyper-geometric (referred to in the Messner and Berger manuscript as the beta-Poisson)—fit the data significantly better than the more complex isolate-focused models (Messner & Berger, 2016a). In terms of statistical fit, these three models were equivalent. The one-parameter fractional Poisson model is the simplest but assumes that all *Cryptosporidium* oocysts used in the studies were capable of initiating infection. The exponential with immunity model does not require that assumption and includes the fractional Poisson as an upper-bound special case. The beta-Poisson model does not include a component for an immune human subpopulation. Importantly, all three models predict substantially higher risk at low doses than do prior models and any of the three could be justified equally from a technical perspective.

Schmidt and Chappell (2016) commented on the Messner and Berger (2016a) manuscript with concerns regarding 1) the unacknowledged assumption that all *Cryptosporidium* isolates share a single dose-response relationship and are therefore equally infectious, 2) the inconsistencies between model assumptions and published clinical experiences (*i.e.*, feeding studies) and 3) statistical evidence contradicting appropriateness of the newly recommended model forms. Messner and Berger (2016b) responded that the great differences in dosing level between isolate studies led to multilevel models that were no better than the simpler model in terms of their statistical likelihood. They also indicated that there are insufficient low-dose data to settle the critical technical matters, and that until low-dose data are obtained, no single model can resolve the underlying uncertainty in low-dose risk of infection estimates. As a result, recent water reuse risk assessments (Pecson et al., 2022a; Schoen et al., 2023) have included the fractional Poisson model, which yields upper-bound risk results in the low dose range of interest (less than 10 oocysts). Given this combination of characteristics, the dose-response parameter values for infection shown in Table 4 refer to the fractional Poisson model because it is one of the models that fit the available data equally well, the model is consistent with recent peer-reviewed QMRA studies for water-related exposures, and the model has the characteristic of being health protective in the low dose range.

Prior to Messner and Berger (2016a), several other infection dose-response relationships for *Cryptosporidium* were used in QMRA studies, including those from Teunis et al. (2002a, 2002b), U.S. EPA (2006c), U.S. EPA (2005), DuPont et al. (1995), Haas et al. (1996; 1999), Englehardt and Swartout (2004; 2006), and Bloetscher et al. (2020a). The Messner and Berger (2016a) work suggests a greater risk of infection at low doses than previously predicted.

For the dose-response illness parameter, the values reported in Table 4 are based on work conducted by the EPA to develop the Long Term 2 Enhanced Surface Water Treatment Rule (LT2 ESWTR) (U.S. EPA,

2003). In that analysis, the reviewed studies showed a wide range of illness rates for the relativity high doses used in feeding studies. For example, DuPont et al. (1995) and Haas et al. (1996) found that 39% of those infected had clinical cryptosporidiosis across administered doses, and Okhuysen et al. (1998) found that 58% of their subjects who received doses of *Cryptosporidium parvum* developed diarrhea. However, the subjects in these three studies were administered doses higher than those that are commonly present in water. Chappell et al. (1996) observed that the rate of diarrheal illness was higher for the TAMU or UCP isolates of *C. parvum* than for the IOWA isolate first studied by DuPont et al. (1995) and Haas et al. (1996). Given these results, the EPA's review in the LT2 ESWTR suggests a lower bound of 30% and an upper bound of 70% (Table 4), which have been used to calculated illness rates in QMRAs for water-related exposures.

Giardia spp.

For the reference pathogen *Giardia* spp., the current infection dose-response relationship is based on work published by Rose et al. (1991a), who reported a quantitative description of the dose-response relationship for *Giardia lamblia* infectivity using experimental data by Rendtorff et al. (Rendtorff & Holt, 1954; Rendtorff, 1954). In these experiments, the exponential model was statistically consistent with the experimental data on infection. The dose-response parameter values for illness (Table 4) are based on a review of the literature reported by Eisenberg et al. (1996) indicating that the proportion of symptomatic *Giardia* infections varies considerably. For example, Lopez et al. (1980) reported a communitywide survey of residents revealing that 76% of *G. lamblia* infections occurring during an epidemic period were asymptomatic (*i.e.*, 24% symptomatic illness). On the other hand, Hill and Miller (2011) estimated that 5%–15% of infected persons become asymptomatic cyst passers (indicating 85% symptomatic illness).

Chapter 6. Risk Characterization for Water Reuse

The NRC conceptual risk assessment framework consisting of hazard identification, exposure assessment, dose-response assessment and risk characterization (National Research Council, 1983) has been used by many researchers as a basis for waterborne enteric pathogen QMRA (Asano et al., 1992; Gerba et al., 1996; Haas, 1983; Medema et al., 2003; Regli et al., 1991; Rose et al., 1991a; Teunis et al., 1997) as well as foodborne pathogen risk assessments (Buchanan et al., 1998; Buchanan et al., 2000; Farber et al., 1996). This chapter addresses the risk characterization component of waterborne QMRA for water reuse applications.

Risk Characterization Approaches

For water reuse risk characterization, assessments typically use the conceptual analytical approach that was first published in the 1970s (Dudley et al., 1976; Fuhs, 1975) and assume that the number of individuals who are susceptible to infection does not vary with time (*i.e.*, remains static) at the population level (Eisenberg et al., 2002). These static models have been widely used in water reuse QMRAs (Chaudhry et al., 2017; Lim et al., 2017; Soller et al., 2017a) and by the EPA in developing drinking water regulations (U.S. EPA, 2002, 2006b). Static models may be implemented numerically in several fundamental ways, the major difference among them being the way in which model parameters are characterized. The simplest approach is to use a single value (point estimate) for each of the model parameters (*e.g.*, ingestion volume, density of reference pathogens, dose-response parameters). The output from this static, point estimate modeling approach is a single-valued risk estimate. For example, the state of California used this approach to derive required reductions of reference pathogens across wastewater treatment for its initial IPR regulations (State of California, 2018).

Another implementation method for a static QMRA model is to use statistical distributions for each (or some) of the parameters to account for their variability and/or uncertainty (*i.e.*, stochastic modeling). This stochastic implementation method requires the use of a mathematical technique called Monte Carlo simulation (Fishman, 1995; U.S. EPA, 1997) to numerically sample the input distributions and calculate associated risk estimates. The output from this modeling approach is an estimate of risk in the form of a statistical distribution, from which a threshold value or values (*e.g.*, 95^{th} percentile) is (are) reported and/or evaluated. Such methods have the advantage of accounting for uncertainty among source data and providing a variety of endpoint estimates for downstream risk management (U.S. EPA, 1992). Numerous examples of this static stochastic approach have been published in the literature (Boehm et al., 2019; Chaudhry et al., 2017; Gerrity et al., 2023; Jahne et al., 2023; Nappier et al., 2018; Schoen et al., 2023; Soller et al., 2018b).

Another implementation approach for a stochastic model is to rely on Bayes' theorem and Markov Chain Monte Carlo methods (Gilks et al., 1996; Hastings, 1970; Metropolis et al., 1953). Unlike the Monte Carlo sampling methods described above, which draw independent samples from the specified statistical distribution(s) in each model iteration, Markov Chain Monte Carlo methods draw samples such that each subsequent sample is dependent on the existing sample (Geyer, 1992). This "history" may be used to conduct detailed sensitivity analyses by directly assessing the model simulation conditions leading to high (or low) risk, and the resulting chains can be used for predicting future parameter states. Additionally, this approach allows for expert judgement-based information to be used to inform starting

conditions (termed prior distributions) and parameter uncertainty assessments. These techniques have been used in waterborne QMRAs (Bloetscher et al., 2020b; Englehardt & Swartout, 2006), including those conducted by the EPA in evaluating the dose-response relationship for *Cryptosporidium* for establishing drinking water regulations (U.S. EPA, 2006c), yet are more computationally complex than conventional Monte Carlo approaches and may be strongly influenced by the assumed prior distributions, when input data are sparse.

In addition to static microbial risk assessment (MRA) models, other models have been developed to include the innate properties of infectious disease processes such as person-to-person transmission of infection and immunity (Eisenberg et al., 1998; Eisenberg et al., 1996; Soller, 2006). Addressing these population-level processes requires mathematical modeling approaches that allow the number of individuals who are susceptible to infection to vary with time (*i.e.*, dynamic QMRA models) (Anderson & May, 1991; Hethcote, 1976; Hethcote, 2000). Dynamic methods have been used for numerous waterborne QMRA studies in the United States (Eisenberg et al., 1998; Eisenberg et al., 1996; Koopman et al., 2002; Soller et al., 2003; Soller et al., 2006) and to support drinking water regulatory decisions by the EPA (U.S. EPA, 2006a). From a modeling perspective, there are trade-offs between biological "realism" and analytical complexity. The increase in the complexity of the model structure increases output variability and computational demands (U.S. EPA, 2004a). On the other hand, a simpler model involves implicit or explicit assumptions that may or may not be realistic or appropriate for a particular situation. Soller and Eisenberg (2008) evaluated representative static and dynamic QMRA models to consider the question of model parsimony and identify conditions under which the more complex dynamic models may provide sufficient additional insight that the added complexity is warranted. The results indicated that (stochastic) static risk characterization models are generally acceptable for water reuse exposures and thus are the preferred approach for these applications.

Forward QMRA Approach

Risk characterization methods may be used quantitatively in several equally valid ways. One common approach is to use the model inputs described above to estimate risk(s) associated with a particular exposure scenario ("forward QMRA"). For example, a forward QMRA may estimate the risk of infection associated with potable reuse of recycled water produced by a particular advanced water treatment facility. In this case, pathogen densities in raw wastewater coupled with estimated or measured reductions across unit treatment processes would be used to estimate a pathogen concentration in the product water, which is used in conjunction with an ingestion estimate to generate an estimate of exposure (*i.e.*, dose). This estimate is then input to the dose-response relationship which generates an estimate of risk of infection during the exposure. For example, Lim et al. (2017) quantified the health risk associated with exposure to *Cryptosporidium* and norovirus through drinking water contaminated with wastewater effluents in the Trinity River, and Soller et al. (2017a) evaluated the potential microbial risks associated with various DPR treatment train combinations for recycled water.

Reverse QMRA Approach to Determine Log_{10} Reduction Targets

Using the same inputs, it is also possible to determine the amount of treatment that would be needed to achieve a predetermined level of risk (*i.e.*, calculate an LRT) (Jahne et al., 2024; Schoen et al., 2017; Soller et al., 2018b). In this "reverse QMRA" approach, the same set of equations is used as in forward

QMRA. However, the level of risk is pre-specified and the required reduction in pathogen concentration to achieve that risk is computed instead. For an infection risk metric (discussed below), the annual probability of infection ($Pinf_{annual}$) is set equal to a specific target level of infection risk, and Eq. 1, adopted from Schoen et al. (2017), is solved for the pathogen LRT:

$$Pinf_{annual} = S * (1 - \prod_{n_i}[1 - DR(V_i * 10^{log_{10}(C) - LRT})]) \quad \text{Eq. 1}$$

where

S is the fraction of people in the exposed population susceptible to each reference pathogen (typically assumed = 1)

DR(...) is a dose-response function for the reference pathogen

V_i is the volume of water ingested per day for the activity set *i*

n_i is the number of days of exposure over a year for activity set *i*

C is the pathogen concentration in the source water

This LRT-generating approach is common when developing fit-for-purpose water reuse treatment targets for state regulations (State Water Resources Control Board, 2018; U.S. EPA, 2023) since the results articulate the level of treatment needed for a particular source of water and end-use application combination.

Risk Characterization Duration

In characterizing the health risk associated with human exposure to microbially contaminated water, an important issue is the duration over which the characterization occurs. For example, drinking water regulations focus on protecting individuals against multiple exposures (*i.e.*, up to 365 daily exposures per year) to water with microbial contamination (Regli et al., 1991). For water reuse applications, exposures throughout the day are typically aggregated to give a total daily dose to which the immune system must respond; however, health risk benchmarks are generally based on annualized risks (*e.g.*, 10^{-4} infections or 10^{-6} DALY ppy). These health benchmarks thus require additional assumptions about the number of exposures that are likely to occur over that time period (Gerrity et al., 2023; Jahne et al., 2023; Schoen & Garland, 2017; Signor & Ashbolt, 2009).

While such annualized risk benchmarks are foundational to overall water reuse system design and assessment needs, the actual risks for individuals are driven by conditions that occur through daily exposures, or even short-duration events (*e.g.*, over the course of hours) (Soller et al., 2018a). Short-duration events may be better evaluated and managed when event-relevant risk targets (*e.g.*, daily) are considered (Signor & Ashbolt, 2009; Soller et al., 2018a). Examples of short-duration, hazardous events in water systems include water distribution intrusions, cross-connections and backflows, inadequate management of reservoirs, improper main and pipe repairs and low-frequency failures in unit processes (*i.e.*, pathogen control barriers), such as loss of membrane integrity or disinfection efficacy (Amoueyan et al., 2019; Antony et al., 2016; Jahne et al., 2023; Pype et al., 2016). To address this issue, California has adjusted its DPR regulations from an annual target risk level (*i.e.*, 10^{-4} infections ppy) to a corresponding daily infection risk target (*i.e.*, $10^{-4}/365 = 2.7 \times 10^{-7}$ infections per person per day [ppd]).

This approach ensures that the annual risk target is also met (State Water Resources Control Board, 2018), but functionally results in a more stringent annual risk level as it is highly unlikely that each of the 365 days would exactly reach the daily risk limit.

Source Water Input for Potable Water Reuse QMRA

A key aspect of QMRA risk characterization is the source of water that is used as input to the assessment (Nappier et al., 2018; Schoen & Garland, 2017). For potable reuse QMRAs where municipal wastewater is the source of water, there are two approaches that have been used for risk characterization: use of untreated municipal wastewater and use of secondary treated effluent.

The secondary treated effluent starting point approach (Colorado Department of Public Health and Environment, 2023; Texas Water Development Board, 2015b) results in lower LRTs than the untreated municipal wastewater approach, as some of the required treatment occurs during conventional wastewater treatment upstream of the reuse assessment (Texas Water Development Board, 2015a). However, it requires a site-specific pathogen monitoring campaign to characterize the variable upstream treatment performance and temporal variance for every project (Gerrity et al., 2023). The potential benefits of this approach include the evaluation of a higher-quality matrix (secondary effluent versus raw wastewater) that may be less prone to background interference, and reduced influence of transient conditions and diurnal variability (Gerrity et al., 2022; Tchobanoglous et al., 2021). Potential disadvantages of the treated effluent approach include uncertainty in defining the required characteristics of the monitoring campaign (*e.g.,* duration and number of samples) for robust characterization and the need to process greater sample volumes to increase method sensitivity (Gerrity et al., 2023). Additionally, uncertainty in the ratio of GC to infectious units following wastewater treatment reduces the interpretability of molecular pathogen detections in treated waters (Gerrity et al., 2023; Jahne et al., 2017), limiting the use of norovirus as a reference pathogen under the secondary treated effluent approach.

The use of untreated municipal wastewater for potable reuse QMRA has been well documented in the literature (Nappier et al., 2018). Pathogen viability is highly variable after treatment and untreated wastewater offers the benefits of a more generalizable input source with sufficient pathogen loading for reliable reference pathogen density characterization. One important consideration with this approach is that densities of pathogens, particularly viral pathogens, are often measured via molecular methods, and the molecular targets (qPCR genome copies) more likely correspond to infectious pathogens in raw wastewater than in treated effluent (Gerba & Betancourt, 2019; Teunis et al., 2020; Thebault et al., 2013). Thus, it is preferred that broadly applicable LRTs are based on reported pathogen densities in untreated municipal wastewater, rather than those in variably treated effluents.

Health Metrics and Benchmarks for Water Reuse

Another important consideration from a risk characterization perspective is the health metric to be addressed. Water reuse QMRAs can use several valid health metrics to evaluate risk.

Infection Health Metric

For drinking water in the United States, microbial risk management has traditionally considered an infection-based health metric (U.S. EPA, 1989, 2006c). A probability of infection risk level (benchmark) of

10^{-4} infections ppy was suggested for drinking water treatment requirements early in the development of the Surface Water Treatment Rule (SWTR) (Macler & Regli, 1993; Regli et al., 1991). In the SWTR, the EPA indicated that public water supplies should provide a greater level of protection than is necessary to avoid outbreaks (citing estimated infection rates of 50 in 10,000 people or greater reported for *Giardia* outbreaks in the United States), and stated that "providing treatment to ensure less than one case of microbiologically caused illness per year per 10,000 people is a reasonable goal." Regli et al. (1991) indicated that the 1-in-10,000 (10^{-4}) annual risk target is also "comparable to other acceptable microbiological risk levels" and cited as support the transcript of an expert panel discussion at the 1987 Calgary Giardia Conference (1988). The risk estimates in that discussion ranged over several orders of magnitude, and the expert panel did not attempt to develop a consensus position on a suitable target for drinking water regulations (Sinclair et al., 2015). Although the 10^{-4} infection ppy health benchmark was never formally adopted into U.S. drinking water regulations, it has been adopted by several states in their potable reuse regulation development (Colorado Department of Public Health and Environment, 2023; State of California, 2018; U.S. EPA, 2023). This same metric has also been adopted, or is being considered, for non-potable purposes by numerous states (National Blue Ribbon Commission for Onsite Non-Potable Water Systems, 2023; Sharvelle et al., 2017).

Annual probability of infection has also been used to develop risk-based treatment targets for onsite non-potable reuse (Pecson et al., 2022a; Schoen et al., 2017; Schoen et al., 2023). For these applications, a range of health benchmarks has been evaluated from 10^{-4}–10^{-2} infections ppy, representing potential involuntary or voluntary exposures based on related metrics for drinking water and recreational water, respectively (Schoen et al., 2017; Sharvelle et al., 2017).

The infection health metric is conservative in that not all infections will progress to illness or negatively affect those exposed. However, this health metric cannot account for differences in disease severity among pathogens (*e.g.*, enteric versus respiratory, self-resolving gastrointestinal illness versus complicated sequelae, antibiotic-susceptible versus resistant), nor be directly compared against other types of risk (*e.g.*, chemical exposures or societal conditions (Murray & Lopez, 1997). Those types of comparisons require a health burden-based health metric as described below (Zou, 2001).

Disability-Adjusted Life Year Health Metric

The DALY metric is a measure of the health burden of illness calculated as the sum of years of life lost (YLL) due to premature death and years of life lived with disability (YLD) from illness (DALY = YLL + YLD) (Murray & Acharya, 1997). The 10^{-6} DALY ppy benchmark has been used by the World Health Organization in setting water reuse and drinking water treatment guidelines, as well as in Australia's risk-based reuse guidelines (National Resource Management Ministerial Council et al., 2006, 2008; World Health Organization, 2006, 2017a, 2017b).

When using a DALY health metric, a QMRA model must include the likelihood of illness expressed as the conditional probability of illness given infection (P(ill|inf)) and the associated health burden of disease expressed as the DALY per case of illness for each reference hazard (Schoen et al., 2023). For norovirus and *C. jejuni*, the P(ill|inf) and infection dose-response relationships were recently re-evaluated using an analytical approach that incorporated both challenge and outbreak data (Teunis et al., 2018; Teunis et al., 2020). In this recent work, the P(ill|inf) was shown to be dose-dependent (*i.e.*, P(ill|inf) increases as

the dose increases). In contrast, the current probability of illness characterization for other enteric reference pathogens (*Cryptosporidium* spp., *Giardia lamblia* and *Salmonella* spp.) is considered to be dose-independent (Boehm et al., 2018; Soller et al., 2015; Soller et al., 2018b; World Health Organization, 2006).

The DALY per case of illness has been estimated for foodborne diseases that include water reuse reference pathogens by subregion of the world (Havelaar et al., 2015). The DALY per case of illness accounts for the severity of a health outcome by using a disability weight in the YLD calculation (*e.g.*, 0.061 for mild diarrhea, 0.445 for Guillain–Barré Syndrome, 1 for death) (Havelaar et al., 2015). The values shown in Table 5 were computed and reported by Schoen et al. (2023) based on the North American estimates presented in Havelaar et al. (2015). For *Giardia lamblia*, they adopted the conservative point estimate of Lagerweij et al. (2020). Adenovirus was not included in the Havelaar et al. (2015) analysis and other data were not readily available to estimate the DALY per case of illness for enteric adenovirus infections.

Table 5. DALY per illness for water reuse reference pathogens.

Reference Pathogen	Units	DALY per Illness[a,b]	Reference
Norovirus	genome copies	PERT(0.001, 0.002,0.003)	Havelaar et al. (2015)
Cryptosporidium spp.	oocysts	PERT(0.001, 0.002,0.003)	Havelaar et al. (2015)
Giardia spp.	cysts	0.003	Lagerweij et al. (2020)
Campylobacter spp.	CFU	PERT(0.02, 0.03, 0.05)	Havelaar et al. (2015)
Salmonella enterica	CFU	PERT(0.02, 0.03,0.05)	Havelaar et al. (2015)

[a] PERT distribution (minimum, mode, maximum) (Johnson et al., 1995).
[b] As reported in Schoen et al. (2023) based on data from Havelaar et al. (2015).

Chapter 7. Water Reuse LRTs

The previous chapters of this document summarized the state of the science for water reuse microbial risk assessment inputs, including key reference pathogens for waterborne QMRA; densities of those reference pathogens in municipal wastewater, graywater, stormwater, combined wastewater, and roof runoff (rainwater); reference pathogen dose-response models and parameter values for probability of infection, conditional probability of illness given infection, and DALY per illness; estimates of ingestion volume for potable reuse, unrestricted irrigation, and non-potable indoor use; and parsimonious risk characterization approaches. The values corresponding to those inputs are provided (Tables 1–5). This chapter presents resulting LRTs using the inputs and the risk characterization approach described previously.

State of the Science Peer-Reviewed LRT Estimates for Water Reuse Applications

The resultant LRTs for onsite non-potable reuse and potable reuse are presented in Table 6 (Jahne et al., 2024; Schoen et al., 2017; Schoen et al., 2023; Soller et al., 2018b). The inputs yielding those LRTs reflect an updated set of enteric water reuse QMRA reference pathogens for untreated municipal wastewater and alternative sources of water, where sufficient data were available, along with their corresponding source densities and current dose-response models (Table 1–Table 5). Additionally, the inputs include a potable ingestion estimate that is consistent with current EPA recommendations (U.S. EPA, 2011, 2019, 2024). Evaluation of both infection and DALY-based health metrics provides a variety of treatment targets aligning with previously used risk management perspectives for water reuse. This allows for consistency with previous risk assessments in the United States (*i.e.*, infection health metric) while also providing an alternative metric for those interested in incorporating the impacts of different diseases (*i.e.*, DALY metric), as is common internationally (World Health Organization, 2006). The resultant LRTs in Table 6 represent the estimated level of treatment required (in $\log_{10}$ reduction units) to yield the corresponding benchmark risk of infection or DALY value, or less, in 95% of the corresponding simulated values.

Table 6. Summary of state-of-the-science peer-reviewed LRTs for onsite non-potable reuse and potable reuse (adapted from Jahne et al. (2024)).

End Use	Source of Water	Norovirus		Adenovirus		*Cryptosporidium* spp.		*Giardia* spp.		*Campylobacter* spp.		*Salmonella* spp.	
		LRT_{INF}	LRT_{DALY}	LRT_{INF}	LRT_{DALY}	LRT_{INF}	LRT_{DALY}	LRT_{INF}	LRT_{DALY}	LRT_{INF}	LRT_{DALY}	LRT_{INF}	LRT_{DALY}
Potable use[a,b]	Untreated municipal wastewater	14.5	12.5	NSD	NSD	10.5	10.0	9.5	8.5	11.0	7.5	9.5	9.5
	Untreated onsite wastewater	14.5	12.5	NSD	NSD	11.5	11.0	10.0	9.0	12.0	9.5	8.0	8.0
	Graywater	13.0	11.0	NSD	NSD	9.0	8.5	8.0	7.0	9.5	7.5	5.5	5.5
	Stormwater (10% wastewater)	13.5	11.5	NSD	NSD	9.5	9.0	8.5	7.5	10.0	6.5	8.5	8.5
	Roof runoff[c]	n/a	n/a	NSD	NSD	NSD	NSD	5.5	4.5	9.0	6.5	8.0	8.0
Unrestricted access landscape irrigation*	Untreated municipal wastewater	10.0	8.5	NSD	NSD	6.5	6.0	5.5	4.5	6.5	4.0	5.5	5.5
	Untreated onsite wastewater	10.5	8.5	NSD	NSD	7.0	6.5	6.0	5.0	7.5	5.5	3.5	3.5
	Graywater	8.5	6.5	NSD	NSD	4.5	4.0	3.5	2.5	5.5	3.0	1.5	1.5
	Stormwater (10% wastewater)	9.0	7.5	NSD	NSD	5.5	5.0	4.5	3.5	5.5	3.0	4.5	4.5
	Roof runoff[c]	n/a	n/a	NSD	NSD	NSD	NSD	1.5	0.5	5.0	2.5	3.5	3.5
Indoor non-potable use*	Untreated municipal wastewater	10.5	9.0	NSD	NSD	7.5	7.0	6.5	5.5	7.5	5.5	6.5	6.5
	Untreated onsite wastewater	11.5	10.0	NSD	NSD	7.0	6.5	6.5	5.5	7.5	5.5	4.0	4.0
	Graywater	9.0	7.5	NSD	NSD	4.5	4.0	4.0	3.0	5.5	3.5	2.0	1.5
	Stormwater (10% wastewater)	9.5	8.0	NSD	NSD	6.5	6.0	5.5	4.5	6.5	5.0	5.5	5.5
	Roof runoff[c]	n/a	n/a	NSD	NSD	NSD	NSD	2.0	1.0	5.0	3.0	3.5	3.5

NSD = not sufficient data to compute LRT; n/a = Not applicable, pathogen not known to be present in source.

* Adapted from Schoen et al. (2017; 2023).

[a] Values shown are for DPR; IPR would require the same total reductions but processes other than the advanced treatment facility could be credited.

[b] Some potable reuse strategies (*e.g.*, managed aquifer recharge) are addressed by the Safe Drinking Water Act and these LRTs may not apply.

[c] Roof runoff LRTs are subject to greater uncertainty than others; refer to Schoen et al. (2017; 2023) for discussion.

There are several notable aspects that underpin the results presented above. First, norovirus is highlighted as the key viral reference pathogen, as it causes more illness than all other known enteric pathogens combined in the United States (Collier et al., 2021; Mead et al., 1999; Scallan et al., 2011). For all sources of water and end uses presented in Table 6, norovirus LRTs are the highest across evaluated pathogens. Thus, including updated dose-response relationships for norovirus infection and illness is an important attribute for water reuse QMRAs and the development of microbial treatment targets. This finding is consistent with prior epidemiological studies (Arnold et al., 2017) and risk evaluations for diverse water sources affected by human contamination (Soller et al., 2010a; Soller et al., 2017b).

The comprehensive inclusion of LRT_{DALY} values using updated morbidity and DALY characterizations (Schoen et al., 2023) is a new development in the United States for water reuse applications that derive from untreated municipal wastewater (Jahne et al., 2024). When comparing LRT_{INF} and LRT_{DALY}, it is noteworthy that the differences between treatment requirements were greater for norovirus and *Campylobacter* spp. compared to other reference pathogens (*i.e.*, the LRT_{DALY} for norovirus and *Campylobacter* spp. are smaller than the LRT_{INF}) due to the dose-dependent illness dose-response models (Schoen et al., 2023). Risk metric selection can thus impact resulting microbial treatment decisions.

Peer-Reviewed Water Reuse LRTs Published Previously

Numerous prior publications have also computed LRTs for water reuse applications. A summary of those publications follows, highlighting which QMRA components or inputs in previous LRT studies differ from those identified in this document. In some cases, those QMRA framework components (*e.g.*, dose-response relationships) were not yet available at the time of publication; in other cases, the authors made alternative choices to those identified here.

Peer-Reviewed Potable Water Reuse LRTs

The current LRT estimates for planned potable reuse applications stem from a series of previously peer-reviewed methods and results (Nappier et al., 2018; Soller et al., 2018a; Soller et al., 2019; Soller et al., 2017a). The reference pathogens evaluated in that series of publications include norovirus, adenovirus, *Cryptosporidium* spp., *Giardia* spp., *Campylobacter* spp. and *Salmonella enterica*. A stochastic, static QMRA methodology was used to estimate infection from reference pathogens through ingestion of DPR product water. Mechanistically, the risk characterization methodology used a Monte Carlo simulation approach to generate a distribution of cumulative annual risks of infection due to all of the evaluated pathogens by 1) calculating reference pathogen-specific daily risk estimates; 2) combining pathogen-specific daily risks to generate cumulative daily risk estimates; 3) combining cumulative daily risks to generate a cumulative annual risk estimate; and 4) repeating the process to generate distributions of cumulative annual risk (Soller et al., 2018b). That work suggested that the 10^{-4} infection ppy benchmark level of health protection is only consistently achieved (95% of the time or greater) at viral $\log_{10}$ reductions equal to or greater than approximately 14 for viruses (Soller et al., 2018b).

There have also been other LRTs published and used by states for potable reuse, including California's IPR regulations (California Division of Drinking Water, 2014), California's DPR regulations (California Division of Drinking Water, 2021), and a series of LRT calculations related to DPR as summarized by Gerrity and colleagues (Gerrity et al., 2023). California's IPR LRTs were based on point estimate values

(maximum reported values) for culturable enteric viruses, *Cryptosporidium* and *Giardia*. The viral LRTs were computed by coupling the culturable enteric virus concentrations with a dose-response model for rotavirus (California Division of Drinking Water, 2014). These calculations resulted in LRTs of 12/10/10 (virus/*Giardia*/*Cryptosporidium*) for IPR in California.

California's DPR LRTs were based on point estimate pathogen calculations for norovirus, *Cryptosporidium* and *Giardia*. The norovirus concentration was based on a rounded maximum reported value from the literature (Eftim et al., 2017), and the *Cryptosporidium* and *Giardia* were the same values used in the IPR LRT calculations. The dose-response model for *Giardia* was the same as used in the IPR LRT calculations, norovirus was based on the upper bound values reported by Teunis et al. (2008), and *Cryptosporidium* was based on the work of Messner and Berger (2016a). The corresponding LRTs were 16/10/11, which were then increased by 4 logs to account for treatment unit process failures, yielding final LRTs of 20/14/15. Pecson et al. (2023) reported an alternative set of LRTs (13/10/10 plus 4 log redundancy) that modify the California DPR approach by including probabilistic pathogen distributions, additional dose-response models, and an adjustment factor for virus density data that accounts for culture method limitations. Colorado adopted the California IPR baseline LRTs (12/10/10) for DPR, but also provided flexibility for interested agencies to pursue a treated wastewater effluent alternative with minimum LRTs of 8/6/5.5 (virus/*Giardia*/*Cryptosporidium*) (Colorado Department of Public Health and Environment, 2023). These are minimum LRTs; the actual values can be higher based on the results of the site-specific assessment, but they cannot be lower. This treated effluent approach was developed in Texas for the consideration of DPR on a site-specific basis, where the source of water used to determine reference pathogen densities is treated wastewater effluent (Texas Commission on Environmental Quality, 2022; Texas Water Development Board, 2015a).

Finally, a series of LRT calculations for DPR were conducted and reported by Gerrity and colleagues (Gerrity et al., 2023). This work has many similarities to the LRT calculations described above, including the consideration of viral, protozoan and bacterial reference pathogens and overall QMRA-based model structure. However, it diverges in several ways, the most significant of which is the treatment of viral reference pathogens. For example, 1) the reference pathogen densities in Gerrity et al. (2023) are based on a monitoring program conducted in California during the COVID-19 pandemic (Pecson et al., 2022b), which likely underestimates norovirus densities compared to non-pandemic conditions (Wigginton et al., 2021); and 2) norovirus LRT values include hypothetical adjustments of genome copies to infectious virus units (GC:IU) based on the ratios observed for other viruses in the study for which culture methods are more readily available (enterovirus and adenovirus). Although data indicate a potentially wide GC:IU ratio range for the other viruses in raw wastewater (Pecson et al., 2022b), this approach to norovirus characterization is inconsistent with findings of the recent dose-response study based on unadjusted molecular measurements from outbreak and feeding studies (*i.e.,* demonstrating a probability of infection given exposure to a single genome copy of 8-28%, depending on the norovirus genogroup) (Teunis et al., 2020). Dose-response models in Gerrity et al. (2023) were consistent with the selections previously made for California's IPR and DPR LRTs, with additional inclusion of the Teunis et al. (2016) model for adenovirus. Overall, the LRTs presented by Gerrity et al. (2023) (LRTs used the 97.4th percentile of the pathogen concentration values, or roughly two standard deviations above the mean)

are similar to the LRTs presented in Table 6 for *Giardia* spp. (10 logs) and *Cryptosporidium* spp. (10 logs) but are slightly lower for norovirus (13.1 logs).

Peer-Reviewed Onsite Non-Potable Water Reuse LRTs

The Australian government first established risk-based LRTs of enteric pathogens for domestic and municipal uses for stormwater, graywater and blackwater (National Resource Management Ministerial Council et al., 2006, 2009). This work introduced important inputs required for onsite LRT development such as estimates of exposure volumes; however, a simple, deterministic approach was applied that did not account for variability in pathogen density. Additionally, the reference pathogens did not include norovirus, as the dose-response was not available at the time of LRT development. In a review of non-potable LRTs by Schoen and Garland (2017), additional gaps were identified, such as lack of consideration of collection scale, sporadic pathogen occurrence, and exposures resulting from misuse or failure events.

These limitations were rectified by the pathogen reduction targets for onsite water reuse developed by Schoen et al. (2017). In this work, a probabilistic approach was introduced to calculate LRTs for the use of untreated onsite wastewater, graywater, stormwater and roof runoff as sources of water for non-potable reuse applications (Jahne et al., 2023). This effort introduced models to capture the pathogen dynamics of onsite collection scales (Jahne et al., 2017) and was updated by Schoen et al. (2023) to include recent dose response models and report LRTs that account for the severity of illness using DALYs. The onsite LRTs in Table 6 derive from Schoen et al. (2023), with minor modifications to align with the practices herein (Jahne et al., 2024).

Other LRT values have also been published for onsite non-potable reuse applications. Reynaert et al. (2024) extended the Schoen et al. (2017) work and evaluated graywater LRTs using the infection benchmark for small- to medium-scale onsite systems across a wider range of reuse applications with different exposure and pathogen density assumptions. The LRTs for irrigation and indoor use following the 10^{-4} infections ppy benchmark are similar to those in Table 6 across pathogens (*e.g.*, 6.8 and 8.7 for norovirus, 4.7 and 6.7 for *Campylobacter* spp., and 3.9 and 5.9 for *Cryptosporidium* spp.).

Additionally, LRTs for infection (10^{-4} infections ppy) for the same set of onsite sources of water were evaluated using alternative source water pathogen characterizations (National Water Research Institute, 2021; Pecson et al., 2022a). In Pecson et al. (2022a), dilutions of municipal wastewater pathogen densities from Pecson et al. (2022b) were used to calculate onsite wastewater, graywater and stormwater LRTs, assuming equivalency between onsite and municipal wastewater collections given limited empirical data (Jahne et al., 2023). To determine onsite non-potable LRTs for viruses, Pecson et al. (2022a) used a combination of inputs, including the consideration of culturable adenovirus and a "synthetic virus" which combined properties of several pathogenic waterborne viruses of the enterovirus group (*e.g.*, culturable strains of poliovirus, echovirus, coxsackievirus) for occurrence and rotavirus for the dose-response function. This work indicated that cultivable adenovirus yielded LRT results essentially equivalent to those of the cultivable enterovirus/rotavirus approach in nearly all cases. Given these observations, the authors selected culturable adenovirus as the reference viral pathogen for LRTs. A comprehensive comparison of the Pecson et al. (2022a) and Schoen et al. (2017; 2020a; 2018) work is provided by Jahne et al. (2023).

Chapter 8. Risk Framework Policy Considerations

Key Policy Considerations

The previous chapters of this document summarized the state of the science regarding inputs and numerical approaches that can establish microbial LRTs for a range of water reuse applications. This document strives to present objective scientific information and data to support informed decision-making in the context of water reuse. However, translating risk-based management into practical implementation requires additional considerations for decision-makers, particularly related to health metrics and associated benchmarks.

Health Metrics

Decision-makers need to determine which of the available health metrics suit their needs for circumstances within their specific jurisdictions. Current health metrics for this fit-for-purpose framework include infection and health burden (DALY), with the infection health metric used as the traditional health-based metric in the United States for state-level water reuse applications. The 10^{-4} infections ppy was first suggested as a potential health goal for drinking water during development of the SWTR (Macler & Regli, 1993; Regli et al., 1991). Although that health goal was never formally adopted into U.S. drinking water regulations, it has been adopted by several states in their development of potable reuse regulations (Colorado Department of Public Health and Environment, 2023; State of California, 2018; U.S. EPA, 2023). This same metric has also been adopted or is being considered for non-potable purposes by numerous states (National Blue Ribbon Commission for Onsite Non-Potable Water Systems, 2023; Sharvelle et al., 2017). The infection metric can be appealing in its simplicity and historical use, but only accounts for rate of infection and not the amount or severity of disease.

The DALY health metric, accounting for disease severity, is used by the World Health Organization and several countries for water reuse applications (National Resource Management Ministerial Council et al., 2006; World Health Organization, 2017b). It is also currently under consideration by states and codes organizations in the United States for onsite non-potable reuse (National Blue Ribbon Commission for Onsite Non-Potable Water Systems, 2023). This approach has the appealing attribute of accounting for severity of disease and allowing risk comparisons against other public health concerns (*e.g.*, chemical risks, societal impacts). It does, however, require additional data to characterize probability of illness and DALY per illness values, and thus encompasses additional uncertainties. DALYs are also conceptually more complicated than infection metrics and may be more difficult to explain to the public. However, DALYs may be useful in cases when highly infectious organisms that result in generally mild (or self-limiting) illness need to be considered alongside infectious organisms with a lower probability of illness but higher consequences (*e.g., E. coli* O157:H7), or if other risk-based comparisons are needed (*e.g.*, versus the chemical risks of disinfection).

A third health metric, the probability of illness, is used for recreational water criteria in the United States based on epidemiology studies (U.S. EPA, 1986, 2012c). However, illness has not been previously used as a metric for water reuse, nor has a health benchmark for this metric been established for these settings.

Health Benchmark Levels

As with the health metric, determining the most suitable health benchmark levels (*i.e.*, 10^{-2} or 10^{-4} infections ppy) and exposure durations (annual or daily) for a given reuse application is a policy decision.

As indicated previously, a benchmark level of 10^{-4} infections ppy has been adopted by several states for potable reuse. Although drinking water is not regulated strictly on a health benchmark basis, it has been inferred that the potable reuse 10^{-4} infection ppy benchmark is more protective than the guidelines for surface waters used as drinking water sources (Messner & Berger, 2016a; U.S. EPA, 2006c). For potable wastewater reuse, risk managers may choose to apply the benchmark based on the risk of individual pathogens (as reported here) or the cumulative risk from pathogens combined (Soller et al., 2018b); corresponding results for the latter are available in Jahne et al. (2024).

For non-potable uses Schoen et al. (2017; 2023) and others (Jahne et al., 2023; Pecson et al., 2022a; Sharvelle et al., 2017) have published LRTs that correspond to the same 10^{-4} infection ppy health benchmark as used for potable reuse. However, LRTs for other benchmark levels (*e.g.*, 10^{-2} infection ppy) also have been proposed with a range of justifications (*e.g.*, voluntary versus involuntary exposures) (Sharvelle et al., 2017). Given the framework and data put forward in this review, alternative LRTs can also be computed for other benchmark levels; Jahne et al. (2024) include 10^{-2} infection ppy LRTs for non-potable use that utilize the same inputs reported herein.

A related policy issue is whether the annual risk benchmark (10^{-4} infections ppy) should be expressed as its equivalent average daily risk level (*i.e.*, $10^{-4}/365$ days = 2.7×10^{-7} infections ppd). This approach has had limited adoption, with the notable exception of the state of California adopting it in its DPR regulations (California Water Boards, 2024; State of California, 2024). The implications of these annual versus equivalent daily risks are discussed in detail by Gerrity et al. (2023). It is noteworthy that adoption of an equivalent average daily risk level results in a more stringent annual risk, since it is unlikely that each day will exactly reach the daily benchmark.

Another related policy issue is the comparison point in the LRT distribution to the health benchmark level. For example, Table 6 presents the estimated level of treatment required to yield the corresponding benchmark risk of infection or DALY value, or less, in 95% of the corresponding simulated values. The 95th percentile has been used as a comparison point for various other related purposes such as drinking water and prior onsite and potable reuse LRTs. However, other percentiles could also be used to derive LRTs.

Site-Specific Data for a Local Utility or State

The data and information summarized in this review are primarily based on peer-reviewed literature. For source water characterization, another option is for local utilities or states to collect pathogen monitoring data that are specific to their jurisdictions or facilities and to use that data to derive water reuse LRTs. A combination of the data presented in this state of the science review (*e.g.,* exposure and dose-response models) and jurisdiction-specific data may then be considered in a QMRA to develop appropriate LRTs. For example, prior to the publication of this review, the state of California developed and supported a wastewater monitoring campaign to facilitate the development of DPR regulations (California Water Boards, 2024). Decision-makers will need to weigh the value and costs associated with

such an effort and determine the representative monitoring scheme (Jahne et al., 2024; U.S. EPA, 2019, 2024).

Additional LRTs for Redundancy or Equivalent Safeguards Against Off-Spec Conditions

The presented LRTs (Table 6) represent calculated minimum pathogen removals and are scientifically derived starting points for developing risk-based treatment decisions. However, these LRTs are largely based on nominal performance and do not explicitly account for off-specification conditions. These off-specification conditions are known to occur, typically are of short duration (*i.e.*, minutes to hours), and can have important impacts on net annual risks (Salveson et al., 2018). Non-potable LRTs for indoor use account for a rare cross-connection event that also protects against other failure types; Schoen et al. (2018) summarize the modeled failure durations, magnitudes and exposure durations at which risk benchmarks may be exceeded. For DPR, where the time between water treatment and exposure is short, the inclusion of redundancy in treatment has been suggested as an approach to ensure health protection during periods of sub-nominal performance (*e.g.*, 4-logs added to LRTs to ensure redundancy against undetected failures) (California Water Boards, 2024; Pecson et al., 2023; Soller et al., 2018a; State of California, 2024). Similar redundancy considerations are acutely notable for onsite potable reuse systems, where their decentralized nature presents additional challenges for operations management, monitoring and oversight (Sharvelle et al., 2017). Decision-makers must consider whether additional LRTs or other safeguards (*e.g.,* an engineered storage buffer with diversion capability) are needed in their jurisdictions for water reuse applications.

Inclusion of Enteric Bacteria LRTs

As noted in Chapter 2, pathogenic enteric bacteria are an important class of reference pathogens to consider during risk-based water reuse management. However, enteric bacteria may be easier to remove than viruses and protozoa due to their relative size and biological composition (Jahne et al., 2023; National Research Council, 2012). Additionally, disinfection processes generally require the same or lower doses for enteric bacteria relative to viral pathogens (Hijnen et al., 2006; WaterSecure, 2017a, p. 17; 2017b, p. 33). Accordingly, the SWTR and California's water reuse regulations assume that treatment for the other pathogen classes also effectively manages the risks of enteric bacteria (California Water Boards, 2023; Macler & Regli, 1992; Pecson et al., 2022a; Regli et al., 1991; State Water Resources Control Board, 2018). Other guidances have included bacteria explicitly, particularly considering alternative sources of water where robust virus treatment or a residual disinfectant may not be required (*e.g.*, roof runoff) (Jahne et al., 2023; Sharvelle et al., 2017). However, enteric bacteria are generally not included in existing pathogen reduction crediting frameworks, presenting practical challenges to LRT implementation (Jahne et al., 2023).

Chapter 9. Water Reuse QMRA Research Needs

Review and consideration of the previously presented material provides a robust and flexible fit-for-purpose QMRA framework that is supported by peer-reviewed literature. A key advantage of the described QMRA framework is that it is adaptable to evolving information regarding model inputs. Hence, the current LRT estimates can be periodically revisited and revised as salient new data are collected. The summary of information provided in this review suggests several notable research needs with respect to deriving LRTs for water reuse applications.

Dose-Dependent P(ill|inf) for Other Reference Pathogens

As indicated in the dose-response chapter (Chapter 5), recent publications have shown P(ill|inf) is dose dependent for both norovirus and *C. jejuni* (Teunis et al., 2018; Teunis et al., 2020). This phenomenon had been previously suggested (Teunis et al., 1996), but most waterborne QMRA studies have adopted a dose-independent approach (Haas et al., 1999) given a lack of definitive evidence on the topic. For example, early *C. jejuni* analyses revealed a non-monotonic dose dependence (Teunis et al., 2005). Nevertheless, the recent analyses raise the question as to whether P(ill|inf) might be dose dependent for the other waterborne QMRA reference pathogens, and whether QMRAs that address illness (*e.g.*, DALY-based) could be updated in this regard (Schoen et al., 2023). Additionally, continued improvement in the understanding of the health burden associated with illness (DALY per illness) for all reference pathogens is needed to ensure that future estimates fully account for impacts on susceptible sub-populations.

Additional Data Collection on Pathogen Densities in Sources of Water

As described in Chapter 3, empirical pathogen data for onsite-collected waters remain extremely limited, necessitating various assumptions and modeling approaches to generate QMRA inputs. Additional measurements of onsite wastewater, graywater, stormwater and roof runoff across multiple sites and conditions (or site-specific assessments) would serve to validate, update or ultimately replace existing models. However, improved method detection limits may be necessary to fully characterize pathogen density distributions (Jahne et al., 2020). Alternatively, data inputs to existing modeling approaches could themselves be updated. For example, additional data on zoonotic pathogens in animal feces could improve the roof runoff contamination model (Schoen et al., 2017) and new approaches to stormwater contamination assessment (*e.g.*, use of human-specific microbial source tracking markers) could improve its pathogen characterization relative to sewage (Alja'fari et al., 2022). While robust studies of municipal wastewater have been conducted (*e.g.*, State of California (WateReuse Research Foundation, 2021)), they remain limited to certain geographic areas and timepoints that may not be broadly representative of nominal conditions in the United States. New data collections also offer methodological benefits, since many of the data points used in current models do not reflect contemporary advances in sample processing and microbiological analysis methods.

Viral Characterization and Dose-Response

There is a current effort in the scientific community to develop a readily culturable quantification method for norovirus in water (Ettayebi et al., 2016; Ettayebi et al., 2021; Jones et al., 2015; Wales et al., 2024). The ability to characterize culturable noroviruses in water would be a significant enhancement from the current science, as it could help resolve questions about viability and infectivity of norovirus in water matrices. In particular, outstanding questions about norovirus viability after water treatment processes could be addressed using a readily culturable method. However, norovirus infection and illness dose-response relationships are based on molecular pathogen characterizations (Teunis et al., 2020), presenting critical challenges for the direct use of culturable norovirus measurements to evaluate risks of infection and illness with existing dose-response data.

Additionally, there is a need to improve understanding of the dose-response relationship for enteric adenoviruses. As noted previously, adenovirus LRTs were not included here due to limitations in the

dose-response relationships; most critically, there is a lack of available data for the types causing gastrointestinal illness via an ingestion pathway (Teunis et al., 2016). New dose-response studies for enteric adenoviruses could revise this approach, particularly considering the biological and treatability characteristics that make them an attractive reference pathogen (Jahne et al., 2024).

Allocating Log Reduction Credits (LRCs) and Monitoring to Meet Log_{10} Reduction Targets

The LRTs described above identify the amount of treatment (in units of log_{10} reduction) needed for each combination of source of water and end use application. However, LRTs are the first of two important components that are required to implement a risk-based framework. The second component includes allocating reduction "credits" to each unit process within a treatment facility. This log reduction crediting process facilitates the design of treatment trains for producing water that can be used for the intended application. Before log reduction credit (LRC) allocations for individual unit processes are adjusted or alternative unit treatment processes are substituted, it is important to ensure confidence and accuracy in the minimum reductions consistently provided by a treatment process (Nappier et al., 2018). It is notable that LRTs and LRCs are both typically based on the high-risk end of their respective probability distributions (*e.g.*, 95th percentile risk estimate and 5th percentile performance), providing a conservative level of protection (Sharvelle et al., 2017; Water Research Foundation, 2023).

Determining LRCs creates important research opportunities, as it is critical to ensure that LRTs are achieved for broad classes of pathogens of concern. For example, this may be achieved by using culturable, treatment-resistant viruses when assessing viral LRCs (Jahne et al., 2024), and may include non-pathogenic microbial surrogates (Nappier et al., 2018; WateReuse Research Foundation, 2013). Log reduction value crediting frameworks for enteric bacteria are generally unavailable and remain an outstanding need for practical implementation of bacterial LRTs in jurisdictions that choose to include them (Jahne et al., 2023).

Additionally, it is important to differentiate between the actual level of pathogen removal and that which may be rapidly and continuously demonstrated through monitoring (Pecson et al., 2017). In lieu of direct pathogen measurements (*e.g.*, enumeration of pathogenic viruses after advanced treatment) which are prohibitively challenging with current technology, surrogates may be used at critical control points to provide continuous evaluation of system performance. In this context, surrogates and associated removal crediting frameworks for water reuse unit treatment processes are needed to LRV.

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