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Technical Support Materials: Developing Alternative Recreational Criteria for Waters Contaminated by Predominantly Non-Human Fecal Sources

Technical Support Materials: Developing Alternative Recreational Criteria for Waters Contaminated by Predominantly Non-Human Fecal Sources

U.S. Environmental Protection Agency
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Acronyms

Acronym	Definition
AGI	acute gastrointestinal illness
AIDS	acquired immune deficiency syndrome
AWQC	ambient water quality criteria
BAV	beach action value
BMP	best management practices
°C	degrees Celsius
CAFO	concentrated animal feeding operation
CAWS	Chicago Area Waterway System
CDC	Centers for Disease Control and Prevention
CFR	Code of Federal Regulations
CFU	colony-forming units
CI	confidence interval
CM	centimeters
CSO	combined sewer overflow
CWA	Clean Water Act
DNA	deoxyribonucleic acid
DQO	data quality objectives
E. coli	*Escherichia coli*
EJ	environmental justice
FFU	focus-forming units
FIB	fecal indicator bacteria
g	gram
GIS	geographic information system
GM	geometric mean
H	high
HCGI	highly credible gastrointestinal illness
HHRA	human health risk assessment
HUS	hemolytic urea syndrome
ID	infectious dose (“identification” in Appendix C)
IEM	integrated environmental modeling
ILSI	International Life Sciences Institute
IQ	intelligence quotient
kDA	kilodalton
L	liter (or “low” in Table 2-4 or “litter” in Table 2-7)
Ln	natural logarithm
LOD	limit of detection
log	logarithm
logSD	log standard deviation
LOQ	limit of quantification
LT2ESWTR	Long Term 2 Enhanced Surface Water Treatment Rule
M	medium
MAC	microbial water quality assessment category
mL	milliliters
MPN	most probable number
MRA	microbial risk assessment
MS	Microsoft
MST	microbial source tracking
NA	not applicable
ND	not detected
NEEAR	National Epidemiological and Environmental Assessment of Recreational Water
NGI	NEEAR gastrointestinal illness
NIST	National Institutes of Standards and Technology
NORS	National Outbreak Reporting System
NR	none reported
OW	Office of Water
PCR	polymerase chain reaction
PFU	plaque-forming units
pH	potential of hydrogen
POTW	publicly owned treatment works
QMRA	quantitative microbial risk assessment
qPCR	quantitative polymerase chain reaction
RBT	risk-based threshold
RNA	ribonucleic acid
RO	reverse osmosis
RP	recursive partitioning
RWQC	recreational water quality criteria
SAP	sampling and analysis plan(s)

SCCWRP	Southern California Coastal Water Research Project
SD	standard deviation
SIC	sanitary inspection category
SOP	standard operating procedure
SRM	standard control material
STEC	Shiga toxin-producing *E. coli*
STV	statistical threshold value
TMDL	total maximum daily load
TSM	technical support material(s)
UF	ultrafilter
UV	ultraviolet
VBNC	viable, but nonculturable
WHO	World Health Organization
WQC	water quality criteria
WQS	water quality standard(s)
WWTP	wastewater treatment plant

1.0 Introduction

In 2007, 43 national and international experts from academia, numerous states, public-interest groups, the U.S. Environmental Protection Agency, and other federal agencies participated in the Experts Scientific Workshop on Critical Research and Science Needs for the Development of New or Revised Recreational Water Quality Criteria ("Expert Workshop") to identify research needs in the area of recreational water quality research and implementation issues. A key recommendation from the Expert Workshop was to develop more flexible recreational water criteria because "one size fits all" criteria would be inadequate for both public health protection and Clean Water Act (CWA) compliance activities (U.S. EPA, 2007a,b). There is recognition that a single indicator measured to gauge water quality may derive from many sources (i.e., humans, animals [agricultural and wildlife], and environmental) and the health risks to humans vary depending on which of these sources is contributing to the indicator being measured and the pathogens that can cause illness. Public health warning systems based on the single indicator approach do not provide an equal level of health risk protection in all recreational waters. Notification and remediation activities, such as beach postings, listings, and total maximum daily load (TMDL) determinations, are not necessarily focused on the recreational waters representing the greatest potential health risk. These activities occur in recreational waters where indicator levels exceeding standards can result from "natural" or environmental sources and processes (U.S. EPA, 2007b).

The experts identified critical path research for supporting the development of new criteria to include provisions that account for potential differences in human health risks associated with human versus nonhuman sources of fecal contamination. As noted in the Experts Workshop Report (U.S. EPA, 2007b):

> It is widely believed that human feces pose a larger health risk than animal feces to swimmers and other primary contact recreational water users. This belief derives from the basic concept that virtually all enteric pathogens of humans are infectious to other humans, while relatively few of the enteric pathogens of animals are infectious to humans…The bottom line is that there are few data to demonstrate whether animal feces pose a lower, greater, or equivalent heath risk to swimmers than human feces. If there is a difference, it would be important to know the magnitude of the difference, in order for EPA to make appropriate criteria recommendations. The only way to get a better sense of the health risk to swimmers posed by animal feces is to conduct targeted studies.

To help address the identified data gap, the experts recommended enhancing epidemiological study designs, using quantitative microbial risk assessment (QMRA) to help characterize potential human health impacts, evaluating loading, fate and transport of pathogens, and developing quantitative sanitary investigations. Experts also ranked agricultural animals (e.g., cattle, poultry, and sheep) and aquatic birds as the highest priority sources to begin assessing the relative risk differential (U.S. EPA, 2007b).

In response to the Expert Workshop report recommendations and the Consent Decree and Settlement Agreement of 2008 (U.S. EPA, 2008a),[1] the EPA developed a QMRA-based framework for application to

[1] The EPA agreed to "conduct QMRA (based on measurement of pathogenic organisms and indicators) to estimate illness at a freshwater beach impacted by agricultural animal sources of fecal contamination." Case 2;06-cv-04843-PSG-JTL Document 159-3 Files 08/08/2008 Page 3 of 15.

recreational waters and analyzed the potential human health risks from various sources of fecal contamination impacting surface waters based on published data for zoonotic pathogens associated with waterborne disease (U.S. EPA, 2007c, 2010a). The QMRA results from this and other follow-on analyses conducted by the EPA and analyses conducted by others demonstrated the potential for a lower mean probability of gastrointestinal illness, by approximately two to four orders of magnitude, for selected animal sources of fecal contamination; also, the physical processes associated with fate, transport, and environmental attenuation of pathogens (see Section 2.1.1.2) can influence the burden of illness among the exposed population (Soller et al., 2010b; U.S. EPA, 2010a; WERF, 2011; McBride et al., 2013; Soller et al., 2015). Therefore, the source, magnitude, and mechanism of the fecal loading to a surface water influences the pathogen profile present and the potential for illness in recreators. A fecal "source" could be human (point or nonpoint inputs) or be a specific animal (e.g., pigs) or group of animals (e.g., wildlife). The magnitude of fecal loading refers to the amount of mass or volume of feces entering a waterbody. The mechanism of fecal loading includes consideration of how the feces reaches a waterbody and the receptor (e.g., dry weather point source discharge, wet weather mobilized manure, direct fecal deposition).

The EPA's 2012 Recreational Water Quality Criteria (RWQC; Section 6.2.2) includes a discussion of deriving site-specific alternative criteria using QMRA (U.S. EPA, 2012). Part of Section 6.2.2 of the RWQC document highlights QMRA as a tool that could be useful for developing alternative RWQC for sites impacted by nonhuman sources (Boehm, 2009; Dorevitch et al., 2010; Soller et al., 2010a,b; U.S. EPA, 2007a,b).

Site-specific alternative water quality criteria (WQC) could be helpful in cases when a waterbody has characteristics that differ from those of waterbodies that the EPA studied when developing the 2012 RWQC. For example, the epidemiological data that informed the development of the RWQC were conducted at beaches predominantly affected by human fecal contamination. This document provides information on how to use a risk-based approach to develop WQC that are equally health-protective as the EPA's 2012 RWQC for waterbodies predominantly affected by nonhuman fecal loading. The alternative WQC developed may be numerically higher or lower than the 2012 RWQC-recommended water quality values and would be scientifically defensible and protective of the recreational designated use. The purpose of alternative WQCs is to reflect local conditions, site-specific characteristics, and local risk management factors that differ from the basis underlying the 2012 RWQC.

In the 2012 RWQC, the EPA recommended using criteria values associated with specific target illness rates. This approach was taken because the EPA had translated the historically accepted target illness rate from the previous definition of highly credible gastrointestinal illness (HCGI) to the newer definition of illness used in the EPA's National Epidemiological and Environmental Assessment of Recreational Water (NEEAR) study, called NEEAR gastrointestinal illness (NGI). The EPA's 2012 RWQC geometric mean (GM) values are associated with a defined NGI rate (Text Box 1). In deriving the 2012 RWQC, the EPA relied on epidemiological data at beaches where human fecal sources, such as poorly treated sewage and secondary-treated and disinfected wastewater effluent, were identified as the predominant fecal source affecting water quality (Cabelli, 1983; Dufour, 1984; U.S. EPA, 1986, 2012; Wade et al., 2003, 2008). Research indicates that human enteric viruses (e.g., adenovirus, enterovirus, and norovirus) are the

Text Box 1-1. RWQC 2012 Target Illness Rates

The target illness rate associated with the recommended national 2012 RWQC is 32 or 36 NGI per 1,000 recreators.

primary disease-causing agent of concern in predominately human-impacted waters (Cabelli et al., 1982; Soller et al., 2010a; Arnold et al., 2013; Colford et al., 2012; Dorevitch et al., 2012).

However, animal feces can contain zoonotic bacterial and protozoan pathogens and waters predominantly affected by animal fecal loading can present less potential for human health risks compared to waters affected by human feces (WHO, 2003; Roser et al., 2006; Schoen and Ashbolt, 2010; Soller et al., 2010b). Differing characteristics of the three main pathogen classes (i.e., bacteria, protozoa, and viruses), such as occurrence, prevalence, and infectivity, influence the potential risk of gastrointestinal illness to those exposed to waters containing differing pathogen profiles. Contamination patterns and mass loadings of fecal material to surface waters can differ for human versus nonhuman fecal sources (e.g., continuous point source discharges, event-driven nonpoint source inputs).

Delineating the potential human health risks that nonhuman sources of fecal contamination pose relative to those risks when the fecal source is predominantly human has been a major area of uncertainty for risk managers and regulators. Therefore, for waterbodies that are not predominantly affected by human fecal sources, the QMRA approach described in this Technical Support Materials (TSM) document may be used to estimate health risks for fecal loading scenarios, including for those scenarios where epidemiological approaches have not been successful or have not been studied. QMRA provides a scientifically defensible approach to characterize potential risks for different fecal loading scenarios. QMRA is a formal process for quantifying or estimating the risk of illness due to exposures to microbes. Through the process of using a microbial risk assessment (MRA) framework to inform human health protection, diverse information pertaining to a specific pathogen or set of pathogens is compiled in a logical, transparent, and scientifically defensible manner. Decision-makers can use the results of QMRA to decide how to protect public health from fecal contamination, including obtaining answers to their specific questions or examining specific exposure scenarios (Petterson and Ashbolt, 2016). This document describes how to use QMRA to adjust the national RWQC based on the sources of fecal indicator bacteria (FIB) and pathogens affecting a waterbody.

1.1 Purpose and Scope

This TSM supports the implementation of the 2012 RWQC using QMRA to derive site-specific alternative WQC[2] for ambient recreational waters in which the predominant contamination is from nonhuman fecal sources (Text Box 1-2). Specifically, this TSM describes a peer-reviewed QMRA-based process for developing alternative WQC that modify the EPA's 2012 RWQC recommended criteria for

[2] The term "water quality criteria" is used in two sections of the CWA, Section 304(a)(1) and Section 303(c)(2). The term has a different program impact in each section. In Section 304, the term represents a scientific assessment of ecological and human health effects that the EPA recommends to states and authorized Tribes for establishing WQS that ultimately provide a basis for controlling discharges or releases of pollutants. Ambient water quality criteria (AWQC) associated with specific stream uses, when adopted as state or Tribal WQS under Section 303, define the maximum levels of a pollutant necessary to protect designated uses in ambient waters. The water quality criteria adopted in the state or Tribal WQS could have the same numerical limits as the criteria developed under Section 304. However, in many situations, states and authorized Tribes may want to adjust water quality criteria developed under Section 304 to reflect local environmental conditions and human exposure patterns before incorporation into WQS. When adopting their water quality criteria, states and authorized Tribes have four options: (1) adopt the EPA's 304(a) recommendations; (2) adopt 304(a) criteria modified to reflect site-specific conditions; (3) develop criteria based on other scientifically defensible methods; or (4) establish narrative criteria where numeric criteria cannot be determined.

predominantly nonhuman fecal source contributions. The QMRA framework is based on the current state of the science, the EPA's FIB recommendations for enterococci or *Escherichia coli* (*E. coli*), and associated enumeration methods (EPA Methods 1600, 1603, or equivalent). In Sections 2, 3 and 4 of this document, additional information about the exposure scenario, descriptions of the reference pathogens, QMRA parameters, such as dose-response functions, information on risk characterization, and examples of the types of useful supporting evidence that can be included is discussed.

Text Box 1-2. Options for Developing Water Quality Criteria

Under EPA's implementing regulations for §303 of the CWA, states must adopt WQC that contain sufficient parameters or constituents to protect the designated use of a waterbody and are based on sound scientific rationale. States may establish WQC for waterbodies or a portion of a waterbody and, therefore, could establish WQC for a specific site. A "site" may be a beach, a waterbody, a particular watershed, or a collection of waterbodies exhibiting similar water quality or fecal loading characteristics. Alternative criteria could characterize a potentially high-risk scenario and be applied more broadly. The applicability of alternative criteria is a science policy decision. The generalizability of the results beyond the study site might require additional information.

The intended audience of this document is risk assessors and those who prepare or review water quality standard (WQS) packages. This document describes the technical steps and explains how risk assessors can perform the analyses needed to develop alternative WQC, including compiling supporting documentation, such as water quality data, and identifying technical and policy decision points for consideration within the TSM approach. This document describes the scientific basis and process for developing the alternative WQC, which can be considered scientifically defensible and protective of the primary contact recreational designated use at the same level of health protection as the 2012 RWQC recommendations. This TSM also allows for straightforward iteration as states revisit and update WQS based on changing needs, scientific advancement, and potential changes in fecal sources contributing to a waterbody.

This TSM document helps users address three main questions:

- Are the predominant fecal contributions to the site from nonhuman sources? See Section 3, Steps 1 and 2.
- What is the potential risk from recreational exposure to feces-associated waterborne pathogens at the site? See Section 3, Step 3.
- How do I calculate alternative criteria to ensure that the proposed site-specific criteria are protective of the primary contact recreational designated use? See Section 3, Step 4.

The EPA recommends first reading the 2012 RWQC (U.S. EPA, 2012), which describes the scientific bases for the national recommended water quality values and the associated target illness rates. For developing site-specific alternative criteria for other indicators and enumeration methods, please see Site-Specific Alternative Recreational Criteria Technical Support Materials for Alternative Indicators and Methods (U.S. EPA, 2014a).

Using the approach described in this document helps to develop a transparent, clear, consistent, and reasonable[3] QMRA for nonhuman fecal sources of contamination. Deriving QMRA-based WQC might also consider policy, regulatory, and other local considerations; these evaluations should be documented in addition to the risk assessment. Transparent documentation of the results in the supporting material for a QMRA-based submission of a new or revised WQS will facilitate the EPA's evaluation.

1.2 Approaches for Evaluating Relative Risks for Nonhuman Fecal Sources

Similar to the EPA's recommended RWQC, recreational water recommendations and guidelines issued by international entities typically rely on epidemiological data collected in waters affected by human fecal contamination (EU, 2006; NHMRC, 2008; U.S. EPA, 2012; Health Canada, 2012; WHO, 2021). There are limited examples of addressing potential risk from nonhuman fecal sources in these recommendations and guidelines, which include indirectly addressing these sources based on a recommended classification and assessment approach (World Health Organization [WHO] and the National Health and Medical Research Council) and directly addressing these sources in guideline development (New Zealand).

The WHO discusses the importance of identifying fecal sources to inform beach classification as part of the recommended sanitary inspection survey (WHO, 2016; WHO, 2021). The inspection survey emphasizes human fecal sources and considers animal sources as generally less important. Australian guidelines also consider human fecal inputs the most important factor in determining the sanitary inspection category (SIC) (NHMRC, 2008). However, nonhuman fecal sources, such as avian fecal inputs, can be associated with elevated FIB levels in a waterbody. Other animal fecal sources, such as those associated with animal husbandry, can contribute zoonotic pathogens to surface waters (WHO, 2021). Both WHO and Australian guidelines discuss the importance of documenting major animal fecal sources as part of the sanitary inspection because zoonotic pathogens potentially present in animal fecal contamination can cause an increased probability of illness in recreators.

The Australian and the WHO guidance documents classify recreational waters using a matrix combining the SIC (i.e., a waterbody's susceptibility to fecal influence) with the microbial water quality assessment category (MAC) (i.e., the 95th percentiles of intestinal enterococci per 100 milliliters [mL]) (Table 1-1). The MAC cut points are defined by the level of excess GI illness risk among bathers exposed to fecally-contaminated recreational water during epidemiological studies (e.g., category "A" corresponds to an expected GI Illness risk <1%) (NHMRC, 2008). The matrix emphasizes human fecal sources relative to nonhuman sources by evaluating a waterbody's susceptibility to human fecal contamination. When a waterbody classified as having a low or very low susceptibility to (human) fecal influence demonstrates elevated enterococci densities (e.g., > 201/100 mL), nonsewage sources of fecal indicators are implied and should be verified by a sanitary survey (NHMRC, 2008). The epidemiological data underlying the MAC are typically collected under fair weather conditions. On the other hand, exceptional circumstances, such as sewer breaks, extreme flooding, and human or zoonotic disease outbreaks, can affect waters in any SIC or MAC. Water quality managers should be aware of potential exceptional circumstances that could affect their waterbodies and what actions to take in response (WHO, 2021).

[3] Transparent, clear, consistent, and reasonable are the underlying principles for good risk characterization. The elements of a sound risk characterization (e.g., key findings, policy choices, uncertainty, and variability) describe, straightforwardly, the critical points that make it valuable in decision-making (U.S. EPA, 2000a).

Table 1-1. Example classification matrix for fecal contamination of recreational water environments* (adapted from NHMRC [2008] and WHO [2021]).

		Microbial water quality assessment category (95th percentiles of intestinal enterococci/100 mL)				
		A **≤ 40**	**B** **41–200**	**C** **201–500**	**D** **> 500**	**Exceptional circumstances[c]**
Sanitary inspection category (Susceptibility to fecal influence)	Very low	Very good	Very good	Follow up[b]	Follow up[b]	
	Low	Very good	Good	Follow up[b]	Follow up[b]	
	Moderate	Good[a]	Good	Poor	Poor	ACTION
	High	Good[a]	Fair[a]	Poor	Very poor	
	Very high	Follow up[a]	Fair[a]	Poor	Very poor	
	Exceptional circumstances[c]	ACTION				

Notes:

* In certain circumstances, there may be a risk of transmission of pathogens associated with more severe health effects through recreational water use. The human health risk depends greatly on specific (often local) circumstances. Public health authorities should be engaged in identifying and interpreting such conditions.

a. Indicates possible discontinuous/sporadic contamination (often driven by results such as rainfall). This is most commonly associated with the presence of sewage-contaminated stormwater. These results should be investigated further, and initial follow-up should include verification of the SIC and ensuring that samples recorded include “event” periods. Confirm analytical results and review possible analytical errors.

b. Implies nonsewage sources of fecal indicators (e.g., livestock), which need to be verified.

c. Exceptional circumstances are known periods of higher risk, such as during an outbreak involving a human or other pathogen that may be waterborne (e.g., avian botulism—where outbreaks of avian botulism occur, swimming or other aquatic recreational activities should not be permitted), or the rupture of a sewer in a recreational water catchment area, etc. Under such circumstances, the classification matrix may not fairly represent risk/safety. Goals for actions associated with these circumstances can prevent exposure and/or remediate the hazard. WHO (2021) discusses approaches for identifying and communicating a predicted or detected hazardous condition and implementing an incident response plan among the relevant agencies involved in order to address a deterioration in water quality.

Risks from nonhuman fecal sources can be influenced, however, by the magnitude of fecal loading. New Zealand’s Microbiological Water Quality Guidelines for Marine and Freshwater Recreational Areas (New Zealand, 2003) document also discusses the use of the SIC and MAC to assess the suitability of a site for recreation but adapted with local health and water quality data. Guidelines for marine waters were informed by epidemiological investigations at seven different types of beaches: two control (minimal fecal impact), two rural (animal waste inputs), and three human-affected (inputs from oxidation ponds) (McBride et al., 1998). The study reported an association between illness and culture-enumerated enterococci, but no statistical difference in illness risk based on the source of fecal material (i.e., human or animal) was identified. Illness risks were significantly lower at the control beaches compared to the other beaches (McBride et al., 1998). The freshwater guidelines were developed based on the results of a water quality study, including monitoring data for 10 fecal indicator organisms and pathogens, and the QMRA (McBride et al., 2002; Till et al., 2008). Twenty-five sites, representing fecal inputs from birds, dairy farming, humans, and sheep, as well as undeveloped/forested areas, across New Zealand were routinely monitored for 15 months. Results of

the risk assessment demonstrated the importance of *Campylobacter*[4] and human adenoviruses as causes of waterborne illness in recreational waters. The authors estimated 4% of all notified cases of campylobacteriosis in New Zealand could be attributed to recreational exposures (McBride et al., 2002). The potential for increased *Campylobacter* infection was used to derive the guideline *E. coli* value for freshwaters (New Zealand, 2003).

Numerous published QMRA examples have been conducted to address gaps in the available data and to aid in decision-making associated with water quality in the peer-reviewed scientific literature. Selected QMRAs from the literature addressing risks from nonhuman fecal sources, deriving risk-based threshold (RBT) values for an indicator or source marker, or using QMRA outputs to inform risk management practices are included in Table 1-2. These examples highlight the utility of QMRA to add additional insight for risk management in waters not represented by traditional epidemiological-based approaches or where it may be impractical to conduct epidemiological studies. Further, combining the use of epidemiological and QMRA approaches can improve the interpretation of empirical health data and provide additional context for risk management decisions (Till and McBride, 2004; Soller et al., 2016) because recreational water epidemiological studies do not characterize the pathogens present in feces-contaminated waters that could lead to illness and, in some cases, epidemiological approaches fail to establish predictive relationships between the FIB and reported illnesses.

In addition to the summarized literature studies included here (Table 1-2), several other EPA resources provide information about MRA, the development of the QMRA framework, and assessing risk from nonhuman fecal sources in surface waters, including:

1. Review of zoonotic pathogens in ambient waters and an overview of the potential risks of animal fecal contamination (U.S. EPA, 2009b).

2. Summary of studies reporting the prevalence of *Cryptosporidium*, *Giardia*, *Campylobacter*, *Salmonella*, and *E. coli O157:H7* in cattle, swine, poultry, other domestic animals, wildlife, and environmental samples (U.S. EPA, 2010a).

3. QMRA to estimate illness at freshwater beaches impacted by agricultural animal sources of fecal contamination (U.S. EPA, 2010a).

4. Summary of the farm factors that influence the presence of zoonotic pathogens in livestock (U.S. EPA, 2010a: Annex 3).

5. QMRA model to evaluate the relative impacts to human health risks from animal-impacted recreational waters (U.S. EPA, 2010a: Annex 2).

6. Microbiological Risk Assessment Tools, Methods, and Approaches for Water Media (U.S. EPA, 2014b) and an interagency Microbial Risk Assessment Guideline Pathogenic Microorganisms with Focus on Food and Water (U.S. EPA and USDA, 2012).

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[4] Both the 1998 and 2002 study reports included sites highly affected by animal fecal loading due to direct deposition and runoff containing animal wastes in the watersheds examined. For example, about 11 million cattle and up to 50 million sheep were resident in New Zealand compared to approximately 3.5 million people (McBride et al., 1998). Sheep are known reservoirs of *Campylobacter* (Yang et al., 2014).

Table 1-2. Selected QMRA examples from the published literature.

Study	Approach	Pathogens included	Indicators included	Relevance
Boehm et al. (2015)	Derived risk-based values for human fecal source markers, HF183 and HumM2.	*Salmonella* spp.; *Campylobacter*; *E. coli* O157:H7; *Cryptosporidium*; *Giardia*; norovirus	HF183, HumM2	Recreational exposures modeled. Target illness rate = 3% NGI. Risk-based approach informs interpretation of data for human fecal source markers, HF183 and HumM2, from water affected by raw sewage. Epidemiological data for the indicators are not available.
Boehm et al. (2018)	Derived a risk-based value for HF183.	*Salmonella* spp.; *Campylobacter*; *E. coli* O157:H7; *Cryptosporidium*; *Giardia*; norovirus	HF183	Compiled decay rate constants for pathogens and indicator into QMRA. Presented the effect of aging sewage contamination on the RBT for HF183.
Brown et al. (2017a,b)	Derived a RBT for the gull fecal marker, *Catellicoccus* (CAT).	*Salmonella and Campylobacter*	CAT	Recreational exposure modeled. Target illness rate = 3% NGI. Addresses nonhuman fecal source, gulls, which can contribute substantially to FIB levels, but not necessarily risk.
Goh et al. (2021)	Derived risk-based values for human fecal source markers in tropical watershed.	Adenovirus, norovirus	Enterococci, human polyomaviruses, *Bacteroides thetaiotaomicron, Methanobrevibacter smithii*	Recreational exposure modeled. Validated specificity and sensitivity of human markers in tropical watersheds. Identified threshold levels of the markers corresponding to 3.6% NGI. Included decay and dilution in modeling.
McBride et al. (2013)	Characterized potential illness risks from exposure to stormwater discharges from human and nonhuman fecal sources.	*Salmonella* spp.; *Campylobacter, Cryptosporidium*; *Giardia*; norovirus, rotavirus, enterovirus, adenovirus	Not reported	Reported risk profiles for children's ingestion exposure. Reported risks associated with short-term, event-based scenarios. Human-associated viruses dominated the predicted risks in discharges. Reference pathogens associated with nonhuman sources had lower risk profiles.
Roser et al. (2006)	QMRA results were used to inform and adapt risk management approaches to reduce bather exposure to fecal pathogens.	*Cryptosporidium, Salmonella, Campylobacter*	*E. coli*, enterococci, fecal coliforms, *Clostridium perfringens,* sulfate-reducing clostridia.	During dry weather, wild birds were predominant fecal sources. Results demonstrated that human sources became notable after > 10 mm rainfall in 24 hours. Results indicated that recreational activities would need to be controlled after larger runoff events due to human contamination.
Schoen and Ashbolt (2010)	Characterized potential illness risk from sewage and gull fecal sources at a recreational beach.	*Campylobacter jejuni, Salmonella enterica,* norovirus*, Giardia intestinalis,* and *Cryptosporidium* spp.	Enterococci	Risks from gull feces much lower than sewage at the same level of indicator. *Campylobacter* dominated the risk from gulls while norovirus dominated the risk from sewage.

Study	Approach	Pathogens included	Indicators included	Relevance
Soller et al. (2010b)	Characterized potential risks from direct deposition of gull, chicken, pig or cattle fecal contamination compared to human fecal sources in a recreational water.	Norovirus, *Cryptosporidium* spp., *Giardia lamblia, Campylobacter jejuni, Salmonella enterica, E. coli* O157:H7	*E. coli*, enterococci	Gastrointestinal illness risks from fresh cattle feces not significantly different from human fecal loading. Fresh gull, chicken, or pig feces were associated with substantially lower risks.
Soller et al. (2014)	Evaluated the influence of multiple sources of enterococci in recreational water bodies on potential human health risk. Compared human and animal sources, human and nonpathogenic sources, and animal and nonpathogenic sources of enterococci.	Norovirus, *Cryptosporidium* spp., *Giardia lamblia, Campylobacter jejuni, E. coli* O157:H7, *Salmonella enterica*	Enterococci	Risks vary with the proportion of enterococci derived from mixtures of human, nonhuman and nonfecal sources. Study reports corresponding enterococci densities for these source combinations equivalent to the target illness rate of the RWQC. Risks are influenced by the potency of the source (e.g., human) rather than the source contributing the most indicator. For mixtures with low human contributions, corresponding enterococci densities could be substantially greater than the RWQC.
Soller et al. (2015)	Characterized potential risks from exposure to freshwater affected by rainfall-induced runoff containing agricultural animal fecal material.	*Cryptosporidium* spp., *Giardia lamblia, Campylobacter jejuni*, *E. coli* O157:H7, *Salmonella enterica*	*E. coli*, enterococci	Risks from animal fecal sources at least an order of magnitude lower than the target illness rate in the RWQC. Equivalently protective enterococci densities in waters receiving these animal fecal sources could be significantly higher.
Till et al. (2008)	Evaluated microbial indicators and pathogens at 25 freshwater recreational and water supply sited throughout New Zealand.	*Campylobacter, Cryptosporidium*, *Giardia,* human enteroviruses, human adenoviruses, *Salmonella*	*Clostridium perfringens, E. coli,* F-RNA bacteriophage, somatic coliphage	*Campylobacter* and human adenoviruses were most likely to cause waterborne illness in recreational freshwaters. Calculated a threshold value for *E. coli* as an indicator of increased campylobacteriosis. Results were used to inform national water quality guidelines in New Zealand.
U.S. EPA (2010a)	Estimated illness in freshwater affected by selected agricultural animal fecal sources.	*Salmonella* spp.; *Campylobacter*; *E. coli* O157:H7; *Cryptosporidium*; *Giardia*; norovirus	*E. coli*, enterococci	Recreational exposure modeled. Target illness rates = 0.8% HCGI. Modeled direct deposition and runoff from land-applied manures. Median risks from animal fecal sources are generally lower than human fecal sources at the same level of FIB. Median risks from direct deposition of cattle manure equal to RWQC illness rate.

1.3 Application of QMRA to Develop Alternative Criteria to Address Nonhuman Fecal Sources

Recreational exposure to waters containing fecal contamination can be a public health concern due to the potential for adverse health effects, including gastrointestinal illness, respiratory illness, and other health endpoints (NRC, 2004; Craun et al., 2005; Wade et al., 2006, 2008, 2010; Fleming et al., 2006, 2008; Parkhurst et al., 2007). Microbial hazards in recreational water contaminated by feces can include pathogenic bacteria, viruses, and parasitic protozoa of human and/or animal origin. Risks to swimmers can differ depending on the source of the fecal loading (i.e., human or animal) because (1) the pathogens in animal manure differ in type, occurrence, and abundance from those in human sewage (WHO, 2004) and (2) the pathways by which human-infectious pathogens of animal origin (zoonoses) reach swimmers can differ from the routes of human enteric pathogens (e.g., intermittent rainfall with mobilization and overland transport as compared to wastewater treatment plant (WWTP) effluent with the relatively constant flow) (U.S. EPA, 2010a).

The potential human health impacts from recreational exposure to waters contaminated by feces have been largely informed by the available health surveys and epidemiological studies since the 1940s and 1950s (Dufour and Schaub, 2007). A common approach in a number of these studies has been to assess whether there is an association between water quality (as measured by culture-enumerated FIB used as a metric of the magnitude of fecal contamination) and reported human health effects. For example, health studies conducted in the United States in the early 1950s provided evidence of a detectable health effect when total coliform densities were observed above approximately 2,300 colony-forming units (CFU) per 100 mL (Stevenson, 1953). In the late 1970s and early 1980s, the EPA conducted epidemiological studies at freshwater and marine beaches primarily impacted by human fecal contamination that demonstrated a positive statistical association between reported health effects over a range of water quality measured by culturable *E. coli* and enterococci (Cabelli, 1983; Dufour, 1984). More recently, the EPA completed the NEEAR study at freshwater and marine beaches primarily impacted by secondary treated and disinfected WWTP effluent (Wade et al., 2006, 2008, 2010; U.S. EPA, 2010c) and examined the associations between rapid, gene-based enumeration methods and incidence of gastrointestinal illness. The sites characterized in these studies were chosen because human fecal sources contain human pathogens that are infective to other humans; thus, an observable effect was expected. Additionally, wastewater treatment more effectively reduces culturable FIB compared to viral and protozoan pathogens and recreational waters receiving this type of human fecal source can have elevated illness at lower levels of FIB (Wade et al., 2006, 2008, 2010, 2022; U.S. EPA, 2007a; Petrinca et al., 2009; Arnold et al., 2016; Rames et al., 2016; Sidhu et al., 2018; Teixeira et al., 2020). Surface waters affected by these human fecal sources, therefore, represent a scenario of one type of public health concern (i.e., human exposure to human-excreted pathogens) and a potentially higher probability of gastrointestinal illness per level of culturable FIB compared to other types of fecal sources.

Due to the differences in pathogen profiles between human and non-human sources and the pathways those sources can take to reach surfaces waters, it can be technically and logistically difficult to conduct epidemiology studies in waters predominantly affected by animal sources of fecal contamination because, but not limited to, nonpoint fecal sources can be associated with wet weather, different fate and transport behaviors between FIB and pathogens associated with nonpoint fecal sources, and beaches without human fecal sources may not visited by high numbers of bathers.

Epidemiological data collected from predominantly nonhuman or nonpoint source-impacted waters have not consistently demonstrated a statistically significant association between water quality, as measured by culturable FIB, and human illness (Calderon et al., 1991; Prüss, 1998; Haile et al., 1999; Wade et al., 2003; Colford et al., 2007, 2012; Dufour et al., 2012). One possible reason is that differences in the source, magnitude, and mechanism of loading of a nonhuman fecal source to a waterbody in the different studies influence the fate and transport of pathogens in the environment and their effect on human illness. For example, waters with high animal waste runoff and direct deposition might have a similar potential to cause human health effects as human fecal-impacted waters (McBride et al., 1998; Soller et al., 2010b). Thus, the lack of a predictive epidemiology-based association between FIB and adverse human health effects does not mean no potential for human health effects exists. Indeed, some epidemiological evidence shows that swimmers report an increased rate of illness compared to nonswimmers in nonpoint source or nonhuman fecal-impacted waters, although the reported illnesses were not statistically associated with the measures of water quality at the beaches studied (Calderon et al., 1991; Colford et al., 2007, 2012; Fleisher et al., 2010; Sinigalliano et al., 2010; Dufour et al., 2012; Arnold et al., 2013). As indicated by this uncertainty, epidemiology-based approaches have mainly been uninformative regarding the extent of health effects in recreational waters receiving nonhuman fecal inputs in the context of the current regulatory framework.

Recreational water epidemiological studies remain costly and can lack the statistical power to assess the potential health risks from recreational exposure to waters affected by nonhuman fecal sources. Epidemiological approaches traditionally require a large number of participants and a detectable difference in the rate of swimmer-associated illness compared to nonswimmers to have enough statistical power to assess associations. Also, if the actual risk of illness due to swimming is low, the epidemiological analysis might not establish a statistical association between illness and FIB.[5] Ethical constraints are also associated with exposing recreators to waters containing known levels of pathogens. Given the reported risk differential between human and some nonhuman fecal sources, the highest-level exposures would likely have to occur on study days with elevated pathogen densities to be detected via current epidemiological study designs. Elevated culturable FIB can still be predictive of fecal contamination or potential human health risks in nonhuman contamination scenarios, but the relationship between illness and indicator can be different, which affects the level of indicator corresponding to the level of public health concern.

MRA is an alternative approach that uses modeling approaches to characterize potential human health risks from recreational exposure to water containing fecal contamination. QMRA is a formal investigative process, analogous to the chemical risk assessment process that the EPA and others use to characterize the nature and magnitude of potential health risks to humans from contaminants that can be present in the environment and to inform decision-making. QMRA is a framework and approach that brings the available information together with mathematical models to address the spread of microbial agents through environmental exposures and to characterize the nature of the adverse outcomes (CAMRA, 2021).[6] As the field of MRA developed, some complexities associated with modeling infectious diseases that are unique to pathogens became clear. Thus, some features of

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[5] FIB provide an estimation of the amount of feces and, indirectly, the presence and quantity of fecal pathogens in the water (NRC, 2004).

[6] An online resource for QMRA includes a QMRAwiki developed by CAMRA at Michigan State University: http://qmrawiki.org (last accessed June 23, 2023).

QMRA necessitate the use of techniques and data in ways that differ from the assessment of chemicals and other risks and include:

- Variation in the ability of individual organisms in a population of pathogens to initiate infection.
- Wide variation in the susceptibility (immunity) of human and animal hosts to infection.
- Wide variation in expression of disease symptoms.
- Risks of secondary (person-to-person) transmission of pathogens.
- Growth of pathogens *in vivo* and, for a subset of pathogens, growth in the environment.
- High variability (spatial and temporal) in the occurrence of pathogens in the environment.
- Difficulty in recovery and enumeration of pathogens.

QMRA can be used to address specific questions that risk managers might have, such as examining specific exposure scenarios that might be of concern. QMRA can be used for a variety of reasons, including to:

- Assess the potential for human risk associated with exposure to human or zoonotic pathogens or both (Soller et al., 2010a,b, 2016, 2017; Schoen et al., 2011; Vergara et al., 2016; Ahmed et al., 2018).
- Evaluate the potential efficacy of mitigation approaches, such as watershed protection measures (Soller et al., 2006).
- Evaluate specific treatment processes to reduce, remove, or inactivate various pathogens (Soller et al., 2003; Schoen and Garland, 2017; Schoen et al., 2017; Nappier et al., 2018).
- Predict the consequences of various management options for reducing risk (Roser et al., 2006; Ashbolt et al., 2010; Viau et al., 2011; Brown et al., 2017a, Soller et al., 2018).
- Identify and prioritize research needs (Ashbolt et al., 2010; WERF, 2011).
- Assist in the interpretation of epidemiological investigations (Soller et al., 2016, 2017).
- Derive WQC linked to a specified level of protection and reflective of specific exposure scenarios and sources of fecal contamination (Roser et al., 2006; Boehm et al., 2009; Viau et al., 2011; Boehm et al., 2015).

Researchers have demonstrated QMRA to be a scientifically valid and effective tool for evaluating and managing the public health impacts of nonhuman fecal sources in recreational waters and for examining the relative pathogenicity of organisms found in animal fecal material (McBride et al., 2002, 2013; Till and McBride, 2004; Roser et al., 2006; Schoen and Ashbolt, 2010; Soller et al., 2010b, 2014; U.S. EPA, 2010a; WERF, 2011). Findings from QMRA relative risk evaluations between waters affected by human and nonhuman fecal contamination have been used to refine the understanding of the lack of health correlations in epidemiological studies in waters predominately impacted by nonhuman fecal contamination. Further benefits of QMRA include the lack of requirement for human subjects and the ability to address risk management for sensitive subpopulations, such as the potential swimming-

associated risk differential between children and adults. Questions related to these areas can be important to risk managers.

In this TSM, QMRA is the general technical approach used to estimate the risk of illness due to recreational exposures to surface waters containing enteric pathogens due to fecal contamination. Additionally, the associated framework discussed can be used as a tool to develop alternative criteria when the sources of FIB are predominantly nonhuman or nonfecal. This framework can be adapted to account for specific microbes, dosage levels, and illnesses of concern, among other parameters. When using this TSM, it is important to completely document collected data, follow accepted practices, and rely on scientifically defensible approaches to be transparent in decision-making.

1.3.1 Summary of QMRA Literature Review

Since the 1990s, the EPA has been collecting and reviewing literature related to QMRA. This effort has included reviewing published information on exposure assessment, human health effects assessment (including dose-response modeling), and risk characterization for waterborne pathogens. The agency also has conducted literature searches to identify studies describing QMRAs or commonly used QMRA techniques relevant to characterizing risks from recreational water exposure and studies describing novel or cutting-edge QMRA-related techniques (Appendix A; U.S. EPA, 2010a). The textbook by Haas et al. (1999, 2014) also provides an extensive overview of the field of QMRA. The EPA has synthesized the information available to evaluate potential risks from animal-derived pathogens (U.S. EPA, 2010a).

The EPA examined the most relevant and highest quality QMRA studies in detail to evaluate the following objectives:

- Develop a list of QMRA studies from which to draw elements of future study designs.
- Identify the pathogens addressed in QMRAs and the potential reasons the study authors selected those pathogens.
- Identify how variability has been addressed in QMRA studies, particularly source variability and consumption/ingestion variability.
- Assess the tendency for risk analysts to include secondary transmission in the estimate of overall risk.
- Compare the practices used by different QMRA researchers and practitioners, particularly sensitivity analyses and risk characterization.

Studies relevant to exposure pathways other than water were not included. For example, high-quality studies in the literature on food were not evaluated in detail, such as studies primarily concerned with post-slaughter processes or the preparation of food products. For example, studies of *Listeria monocytogenes* growth in delicatessen meat storage are not directly relevant to recreational waterborne exposure and were excluded. However, several studies providing novel techniques for incorporating the growth of *Listeria monocytogenes* into exposure assessment are included. Drinking water studies focused on the treatment process in determining risk were excluded as not relevant; however, several studies that assessed the role of source water quality in finished drinking water risk were included.

Based on the studies reviewed, some general observations about the use of QMRA were noted. First, the utility of QMRA has been demonstrated in a wide variety of scenarios, including ambient waters receiving human and nonhuman fecal contamination and the nonfecal contribution of FIB (Till and McBride, 2008; Schoen and Ashbolt, 2010; Soller et al., 2010b, 2015; de Man et al., 2014). QMRA has been used to characterize risk in scenarios where risk estimation by other techniques, such as epidemiological methods, has been difficult to conduct, not yet conducted or would be cost-prohibitive to conduct. Studies by McBride et al. (2004, 2013), Roser et al. (2006), Schoen and Ashbolt (2010), Soller et al. (2010a,b, 2014, 2015), Sunger et al. (2019), Till and McBride (2008), Timm et al. (2016), Vegara et al. (2006), Viau et al. (2011b), and Wong et al. (2009) are examples of QMRA used to address risk management questions and support decisions related to understanding important aspects of waterborne illness. QMRAs typically can evaluate variability when assessing potential health hazards. For example, many of the QMRAs reviewed for this report accounted for the variability in pathogen or indicator density by treating them as stochastic variables.

Several observations can be drawn from the review and comparison of the identified QMRA studies. First, the identified studies focused on a small subset of the pathogens potentially important in waterborne exposure during recreation. The two pathogens analyzed most frequently, rotavirus and *Cryptosporidium*, may be important contributors to the risk of gastrointestinal illness, primarily due to their low infectious dose $(ID)_{10}$ (or another measure of low-dose infection), frequent occurrence in sewage, and, particularly for *Cryptosporidium*, relatively high persistence in environmental matrices. One potential reason for frequent selection of rotavirus and *Cryptosporidium* is that the available peer-reviewed dose-response models are based on oral ingestion. Numerous studies (Petterson and Ashbolt, 2001; Ottoson and Stenström, 2003; Soller et al., 2003, 2006; Eisenberg et al., 2004, 2008; Hamilton et al., 2006; Bastos et al., 2008; Katukiza et al., 2014; Ahmed et al., 2020) used rotavirus as a surrogate for enteric viruses. When considering the potential illness risk posed by enteric viruses to the recreating general population, this approach can be considered health-protective, given that rotavirus is routinely found in sewage and has a high probability of a single organism initiating infection given known dose-response relationships. The norovirus dose-response model has also been used more frequently in recent years (Soller et al., 2010a,b, 2014; Eregno et al., 2016; Murphy et al., 2016; Chaudhry et al., 2017; Ahmed et al., 2018; Simhon et al., 2020; Jahne et al., 2023) and is notable because it is based on doses in units of genome copies (polymerase chain reaction [PCR] based units) (Teunis et al., 2008a, 2020). Soller et al. (2010b) evaluated the Teunis et al. (2008a) norovirus dose response to understand the potential etiologic agents causing the reported illness in epidemiological studies conducted on the Great Lakes; they found it represented the empirical results well. Although the focus of this TSM is for recreational waters that are predominantly affected by nonhuman fecal sources, some loading of human fecal contamination can occur and be accounted for in the QMRA framework by including human-related reference pathogens, such as enteric viruses. However, as discussed in Section 3, the potential risk of gastrointestinal illness can be quickly driven by the human source(s) as the proportion of human inputs increases (e.g., greater than approximately 30% of fecal loading attributable to humans). Waterbodies receiving treated and disinfected effluent would likely not qualify for alternative criteria using the process described in this TSM due to the particular risk profile associated with this source (Soller et al., 2010b, 2014; Schoen et al., 2011).

The review of the scientific literature indicates that the exponential and approximate beta-Poisson models are two commonly used dose-response models. The exact beta-Poisson relationship (i.e., the hypergeometric function [Haas et al., 1999]) was seldom used because it is complex and computationally difficult, nor were empirical models popular in food dose-response studies (e.g., as

described in Buchanan et al. [2000] and Moon et al. [2004]). A disadvantage of the exponential and approximate beta-Poisson models is that they often do not account for variability and uncertainty in dose-response model parameters. There is a need for dose-response models corresponding to different exposure routes (e.g., ingestion, inhalation) because some waterborne pathogens (e.g., adenovirus) can initiate infection via multiple routes. Variability in dose-response model parameters or the response of the exposed population is rarely considered or addressed. A likely cause for the latter is that dose-response model studies do not consistently provide confidence intervals (CI) for model parameters and seldom present quantitative information on the shape of the distribution for parameter estimates.

The review also indicates pathogen density can be relatively straightforward to model. Modeling variability in pathogen density appears to be limited by the scarcity of available data. Among the studies reviewed for this report, many studies employed point estimates for pathogen density. The two most common methods for accounting for pathogen variability among the studies are (1) the use of empirical distributions for pathogen density based on relatively short time series and (2) the assumption of lognormal distribution of pathogen densities. Among studies using distributions to describe pathogen variability, normal, triangular, lognormal, negative binomial, uniform, and Poisson distributions were the most common.

Another finding from the review of the scientific literature is that secondary transmission and (temporary) immunity are often not included in risk estimation. Several studies (Eisenberg et al., 1996, 1998; Soller et al., 1999, 2003, 2006; Koopman et al., 2002) have demonstrated that consideration of secondary transmission and immunity can, in some cases, significantly influence overall risk associated with exposure to pathogens. The scope of this TSM focuses on primary contact recreation; it does not include secondary transmission in the health modeling because the potential risks are characterized, and the guideline values for recreational waters are recommended for recreators engaging in primary contact recreation. Risk managers are tasked with determining whether a waterbody is safe for recreation.

1.3.2 Overview of the TSM Framework

This TSM includes a multi-step decision process (see Figure 1-1) that can be used to determine whether the predominant source(s) of fecal contamination affecting a waterbody are nonhuman and, if so, how to apply QMRA in developing site-specific alternative criteria for recreational waters. The steps in this TSM also include risk management and policy decisions to help plan and refine water quality studies to support decision-making.

The process starts with acquiring information on the sources and loadings of fecal contamination to a waterbody of interest to determine whether it is predominantly affected by nonhuman fecal sources or if the human contribution is sufficient for risks to be comparable to the human contamination scenario characterized in epidemiological studies informing the EPA’s 2012 RWQC. Four major steps comprise the overall process. At each step, evaluating the information collected and analyzed will help address specific decision points for the risk manager to consider when proceeding to the next step.

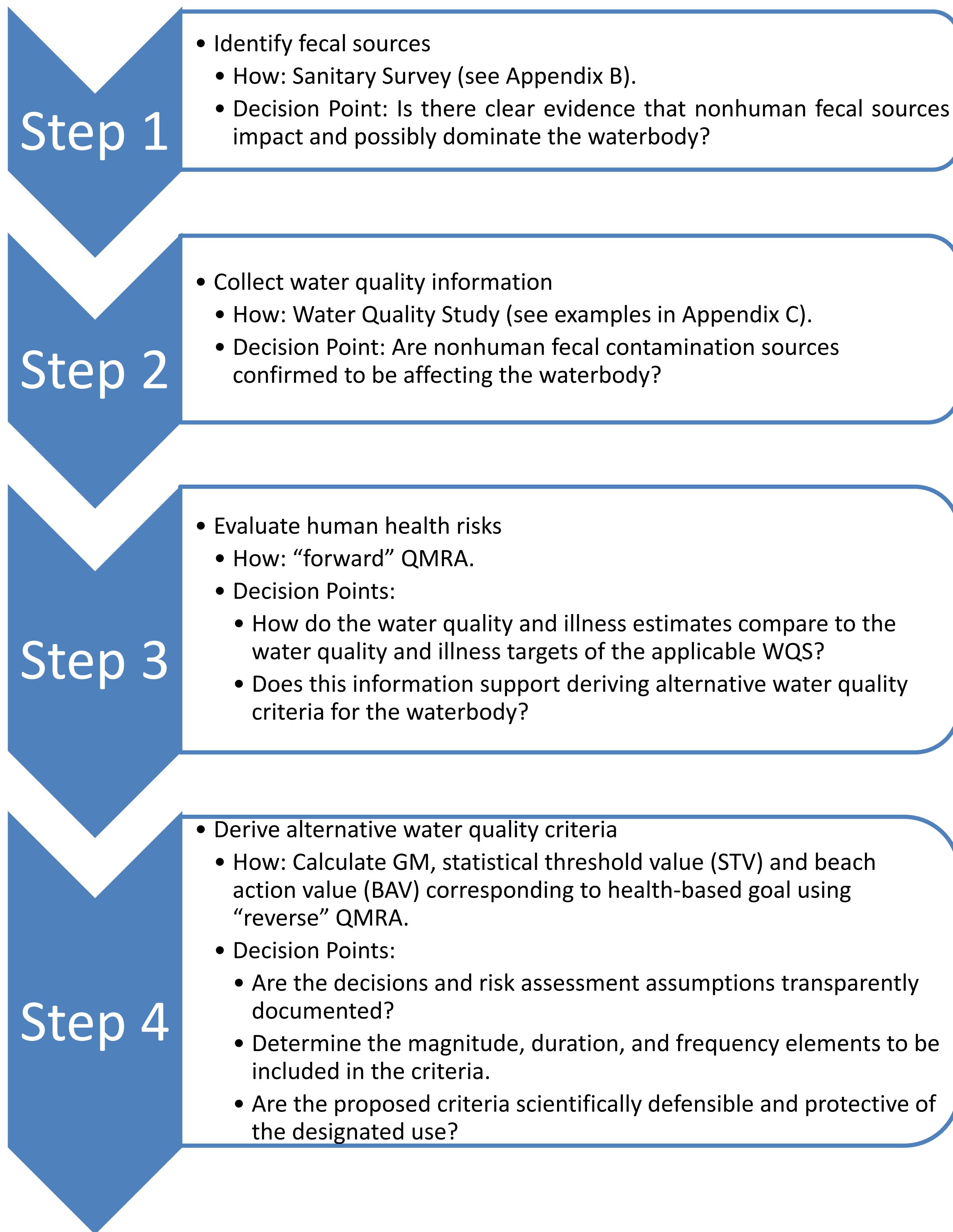

Figure 1-1. Flow diagram for considering QMRA in developing site-specific alternative criteria.

Step 1 consists of identifying the fecal sources in the watershed that can affect the waterbody to determine if QMRA is appropriate. Typically, this is accomplished by using a standardized sanitary survey to determine the sources of fecal contamination. If the sanitary survey findings support the original premise that human sewage does not dominate the fecal contamination entering the waterbody, proceed to the next step.

Step 2 involves collecting water quality data on the waterbody, including information about fecal indicators, pathogens, and fecal source identification and tracking. This information will help characterize the extent of the fecal loading in the waterbody. Steps 1 and 2 together comprise the "Sanitary Characterization," which can provide substantive lines of evidence to support moving forward with conducting a QMRA and potentially developing site-specific alternative criteria. If the sanitary characterization reveals that the waterbody's susceptibility to human contamination is low, such as the absence of significant human fecal loadings, then a decision to proceed to the next step is supported. Lines of evidence supporting a categorical determination of "low" susceptibility to human contamination are also provided in Step 2.

Step 3 consists of evaluating human health risks from recreational exposure to the waterbody. These risks are characterized using a forward QMRA process and the water quality information collected from the watershed. A forward QMRA uses pathogen occurrence as an input and calculates the probability of illness. The results of the QMRA analysis in this step will quantify the potential human health risk from recreational exposure in the waterbody. Conducting a relative QMRA analysis may be useful for interpreting the results of the forward QMRA and should include the same target illness rate associated with the applicable WQS or the EPA's RWQC.

Step 4 describes how to derive alternative WQC and calculate adjusted GM, statistical threshold value (STV) and BAV values for culture-enumerated enterococci or *E. coli* corresponding to the target illness rate.

The EPA also provides a WQS submission checklist to help the user assemble the information that the EPA will use to evaluate a WQS submission (Appendix D).

2.0 Scope and Problem Formulation: Characterizing Human Health Risks from Nonhuman Sources of Fecal Contamination in Recreational Waters Using QMRA

While the generalized risk assessment conceptual framework used in chemical risk assessment can be applied to MRA, differences between chemical contamination and microbial contamination require specific approaches to assessing risk from human infection and resulting illness following exposure to pathogens. To address this need, the EPA's Office of Water developed a conceptual framework in conjunction with the International Life Sciences Institute (ILSI) to assess the risks of human infection associated with pathogenic microorganisms (ILSI, 2000). The purpose of the ILSI framework was to describe a generic approach to identifying scientific information that should be considered when quantitatively assessing human health risks associated with exposure to infectious agents in water (ILSI, 2000). The framework supports the conduct of MRA for various types of microorganisms under various exposure scenarios. The U.S. Department of Agriculture's Food Safety and Inspection Service and the EPA developed the Microbial Risk Assessment Guideline: Pathogenic Microorganisms with Focus on Food and Water ("MRA Guideline") as a resource for developing MRAs (U.S. EPA and USDA, 2012). The MRA Guideline document discusses a flexible framework including general fundamental risk assessment principles that specifically address microbial risks. In 2014, the EPA published Microbial Risk Assessment Tools, Methods, and Approaches for Water Media ("MRA tools") to facilitate the conduct of MRAs for water-related media (U.S. EPA, 2014b, Ichida et al., 2016). The MRA tools document describes a flexible human health risk assessment (HHRA) framework that can be used to inform public health decisions related to microbial hazards in various water-based media. Users can adapt, modify, or tailor the elements within the framework to address specific risk management questions (U.S. EPA, 2014b). These framework documents support risk assessors in developing consistent, high-quality evaluations of microbial risks and informing decisions focusing on the risks posed by infectious microorganisms. The approaches described in these documents help risk assessors characterize exposure sources, causative agents (including naturally occurring pathogens and pathogens introduced via contamination), contributing immunity factors, associated symptoms, acute disease and chronic sequelae endpoints, and potential control points to reduce risk. The EPA's HHRA framework, Framework for Human Health Risk Assessment to Inform Decision Making ("HHRA framework") (U.S. EPA, 2014c), was developed to support risk-based decision-making to maximize the utility of risk assessment. The HHRA framework discusses the importance of the design of risk assessments in the decision-making process and integrates planning, scoping, and problem formulation with traditional risk assessment approaches to support a "fit for purpose" assessment. The approach discussed in this TSM incorporates aspects of the MRA and HHRA frameworks (Figure 2-1). The QMRA analyses described herein are designed to quantitatively assess risk from enteric pathogens associated with fecal contamination in surface waters. The MRA steps are integrated into a decision-based process for risk managers to utilize when addressing questions about the risks posed by nonhuman fecal contamination of surface waters.

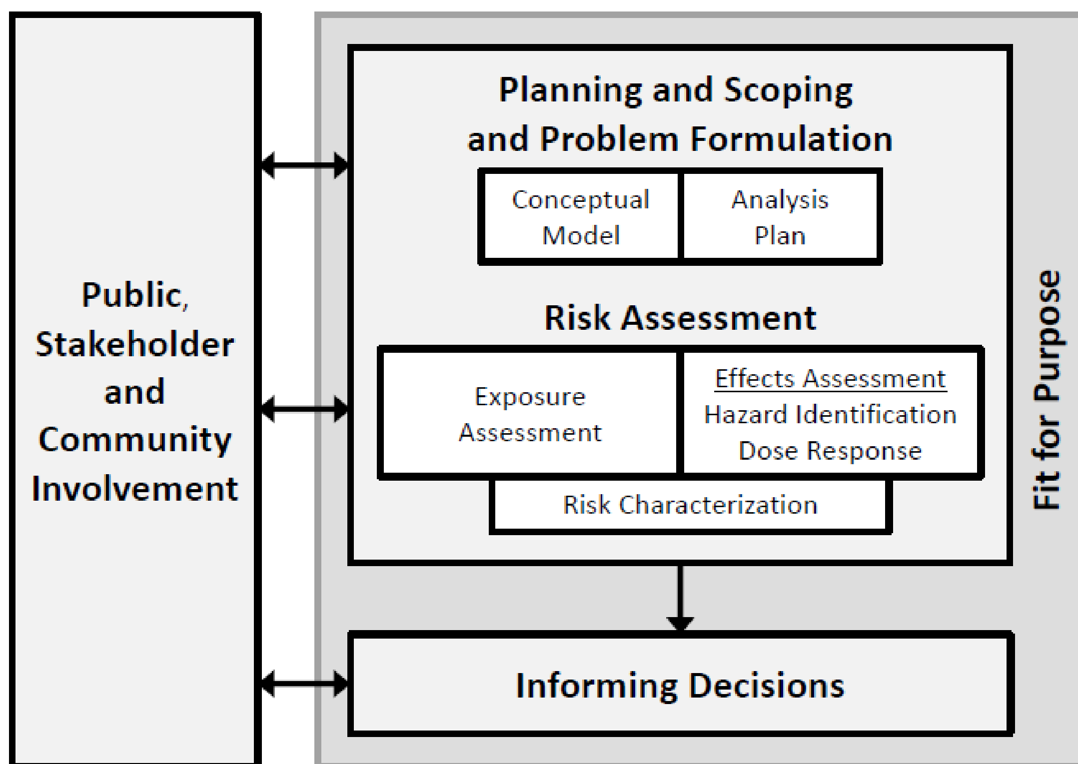

Figure 2-1. Framework for HHRA to inform decision-making (U.S. EPA, 2014c).

The planning, scoping, and problem formulation stage is used to identify: (1) the purpose of the risk assessment, (2) the critical issues to be addressed, and (3) how the results might be used to protect public health. As part of the initial planning process, key stakeholders interested in recreational water quality and safeguarding the public from pathogen pollutants in waters where people recreate can be identified and invited to participate. Stakeholders may include public health officials, the business community, environmental groups, and landowners. Stakeholder input can help define the scope of the effort and ensure that various science, policy, and management perspectives are incorporated into steps of the alternative criteria evaluation process. Throughout the process discussed in this TSM, periodic stakeholder engagement can be used to define the "fit for purpose"—or suitability and usefulness of the activities—for informing risk management decisions. Stakeholder input can also help inform the various decision points identified in this TSM, including when developing and conducting both the sanitary survey and the water quality study supporting the conduct of a QMRA.

Problem formulation is a process for generating and evaluating preliminary hypotheses about why human health effects have occurred or might occur as the result of exposure to sources of human and nonhuman fecal contamination. The scoping and problem formulation steps include the development of the conceptual model and analysis plan and are part of the framework described in this document (Section 2.2). The framework presented in this document also incorporates risk assessment purposes, including to:

1. Establish and quantify the potential human health risk from recreational exposure to waters receiving predominantly nonhuman fecal contamination,

2. Compare the potential risk differential to exposure to human fecal contamination; and,

3. Translate the potential risk differential into adjusted values for culturable *E. coli* or enterococci that are protective to the same level as EPA's 2012 RWQC recommendations.

Users of this document may include additional or updated elements, such as alternative health endpoints, alternative or additional pathogens, or new modeling information. All parameter and model choices discussed in this document are documented in a transparent fashion. The QMRA sanitary survey form (see Appendix B) or portable device application aids users in delineating fecal sources. An example Sampling and Analysis Plan (SAP) describing the process for generating water quality data to inform the QMRA is included (see Appendix C).

During the risk assessment process, information about the exposure to and health effects of fecal-associated pathogens is compiled and summarized. The quantitative and qualitative evaluation of these data, expert opinion, and other information are integrated into the risk characterization, including the associated assumptions and uncertainties. Using QMRA to describe human health risks and inform alternative WQC is a data-driven exercise—from conducting a sanitary survey and characterizing water quality to estimating potential risks and translating risks into alternative indicator values. The QMRA framework allows consideration of specific risk management questions involving sensitive subpopulations, such as the potential swimming-associated risk differential between differing age groups, such as younger children, older children, and adults. Importantly, decisions made during the process discussed in this TSM should be supported by data that characterize the potential sources of fecal-associated pathogens in a waterbody that have the potential to adversely affect human health. Documented data analysis and interpretation support the scientific defensibility and effectiveness of alternative WQC. Iterative engagement with stakeholders to discuss the QMRA parameter selections and results is recommended.

The QMRA framework described in this TSM incorporates the state of the science for QMRA and aspects of previous analyses conducted by the EPA and others (Ashbolt et al., 2010; Schoen and Ashbolt, 2010; Soller et al., 2010a, b, 2014, 2015, 2016; U.S. EPA, 2010a; WERF 2011; McBride et al., 2013; Sunger et al., 2019). Consistent with previously published QMRAs, the QMRA scenario discussed in this TSM considers recreational events as independent events and are treated probabilistically. The independence of recreational events means that the probability of an adverse health effect on one day is not influenced by recreational events that occurred on prior days. To evaluate recreational events probabilistically, statistical distributions, rather than point estimates, of the QMRA input parameters were used. The probabilistic approach produces different model results each time the model is run due to the effects of chance. Each time the QMRA model is run, a random value within each input parameter distribution is used; this process runs for thousands of iterations to obtain the result, which is also a distribution.

Conducting a QMRA in recreational water requires four data inputs:

1. Volume of water ingested during recreational activities,

2. Density of reference pathogens in the ingested water,

3. A quantitative relationship between the number of pathogens ingested (dose) and the probability of infection (dose-response relationship), and

4. The conditional probability of illness given infection (probability of symptomatic response).

These parameters and how they are used in QMRA to estimate the probability of illness in a waterbody using locally collected data are described in this document. Outputs from the data analysis and quantitative health models can support understanding the risk of gastrointestinal illness in the waterbody studied (Section 3.3) and provide the basis for adjusting the EPA's 2012 RWQC recommendations due to the nature of the source(s) of fecal contamination (Section 3.4). Additional information on susceptible subpopulations and sensitivity analyses can be found in Section 4. The discussion in Sections 3 and 4 can help stakeholders integrate and interpret the QMRA-based outputs, characterize uncertainty, and support decision-making.

2.1 Rationale for use of Stressors and Surrogates

Human and animal fecal waste can contain a wide array of pathogenic microbes from three main pathogen categories: viruses, bacteria, and protozoa. The level of each pathogen type can vary in the host and in the receiving waters due to dilution, decay, and other factors (Teixeira et al., 2020). Some of these pathogens are host-specific (e.g., human enteric viruses), while other pathogens can infect and cause illness in both animals and humans (i.e., zoonotic pathogens) (U.S. EPA, 2009b). Fecal contamination of surface waters can result in the transmission of these pathogens, causing waterborne disease (Savichtcheva and Okabe, 2006). Sources of fecal contamination can include human and nonhuman contributions and be categorized as "point source" (e.g., raw sewage, treated effluent from WWTPs, etc., characteristically discharged from discrete and/or permitted sources) or "nonpoint source" (e.g., runoff from agriculture, wildlife, and urban land usages) inputs that can adversely affect water quality. This latter category may enter waterbodies as overland flow, via drainage ditches and creeks, or through less-regulated stormwater conveyances. Animal fecal inputs to surface waters can range from wildlife and vermin sources to animal husbandry operations (e.g., concentrated animal feeding operations [CAFOs]) and agronomic applications of manures.

Plowright et al. (2017) describe "zoonotic spillover" as the transmission of a pathogen from animal to human. Spillover transmission depends on multiple dynamic processes and host interactions that can result in zoonotic infection and illness in humans. The probability of the spillover occurring is influenced by three functional phases: pathogen pressure, pathogen exposure, and human host characteristics (Plowright et al., 2017). First, the presence of pathogens in feces depends on their occurrence in infected individuals in a population and the rate of shedding. Once shed by the animal, pathogens can be disseminated in the environment and can be attenuated by numerous environmental factors. Some excreted pathogens further develop in water as part of their natural lifecycle or find a new host (U.S. EPA, 2009b). Second, human behavior can influence pathogen exposure, including the route and dose of exposure. For the third phase, Plowright et al. (2017) describe the human host's genetic, physiological, and immunological attributes that, given a sufficient dose, affect the probability and severity of infection and resulting illness. Other studies suggest the potential for attenuation of the infectivity, virulence, and disease severity to humans from animal-derived ("passaged") zoonotic pathogens (U.S. EPA, 2009b).

The diversity of pathogens that can be present, typically at low densities in environmental waters, and the challenges associated with frequent, widespread monitoring for those pathogens has resulted in the use of FIB as surrogates for fecal contamination, such as culture-enumerated *E. coli* and enterococci, to routinely evaluate water quality (Schwab, 2007; Korajkic et al., 2018). Pathogen occurrence in surface waters can be variable and their enumeration methods can be complex, labor-intensive, technically challenging, and in some cases not feasible (Savichtcheva and Okabe, 2006;

Korajkic et al., 2018). Not detecting a particular pathogen does not indicate the absence of other pathogens (Ahmed et al., 2018). Because pathogens in the surface water matrix can be associated with high temporal and spatial variability, further complicated by enumeration method sensitivity and variability, the lack of detection may not indicate the absence of the microorganism, just that the level of the target could be below the limit of detection (LOD)/limit of quantification (LOQ). Frequent nondetects or results below the LOQ can be challenging to interpret quantitatively.

In lieu of monitoring for all pathogens that may be present, FIB, such as *E. coli* and enterococci, are routinely enumerated with simple, straightforward culture-based laboratory methodologies. Although FIB can have environmental, nonfecal sources, elevated quantifications above background, especially during baseflow conditions, can be considered indicative of fecal contamination and, indirectly, of the potential presence of pathogens. Traditional culturable FIB are not well correlated with the presence or absence of specific pathogens in environmental waters; however, published epidemiological examples provide statistically significant associations between increasing FIB levels and increases in illness, mainly in waters receiving human fecal contamination (Cabelli, 1983; Dufour, 1984; Kay et al., 1994; McBride et al., 1998). In waters affected by animal fecal sources, the epidemiological evidence is inconsistent, but participants in those studies reported increased illness given recreational exposures (Calderon et al., 1991; McBride et al., 1998; Sinigalliano et al., 2010). Calderon et al. (1991) attributed the reported illnesses to swimmer-to-swimmer transmission due to illness being associated with high numbers of swimmers per day and elevated levels of staphylococci. McBride et al. (1998) observed no differences in reported HCGI at marine beaches receiving animal waste inputs or human effluents from oxidation ponds. Sinigalliano et al. (2010) found an increase in self-reported gastrointestinal, respiratory, and skin illnesses among bathers compared to nonbathers in nonpoint source subtropical recreational marine waters.

The EPA published a review of potential human illnesses caused by recreational exposure to water contaminated by animal feces (U.S. EPA, 2009b). The available recreational water monitoring data, public health reporting, epidemiological studies, illness outbreak reports, and dose-response studies suggest that a subset of pathogens is representative of the majority of hazards due to recreational exposure to human and animal feces-impacted recreational water (Scallan et al., 2011a,b). The review focused on warm-blooded animals (mammals and birds) whose fecal material is detected by currently recommended FIB. Seventy pathogens from warm-blooded animals were evaluated for the following attributes:

1. The pathogen spends part of its lifecycle within one or more warm-blooded animal species.
2. Within the pathogen's lifecycle, it is probable or conceivable that some lifestage will enter water.
3. Transmission of the pathogen from the animal source to human is through a water-related route.
4. The pathogen causes infection or illness in humans.

Twenty pathogens have been shown to possess all four of these attributes. Based on their relevance in the United States, six of these 20 waterborne, zoonotic pathogens from warm-blooded animals were selected for further evaluation. Five of the six were linked to illness outbreaks in ambient, untreated recreational water, and the sixth was included based on outbreak reports in drinking water (U.S. EPA, 2009b). The six key waterborne zoonotic pathogens identified in the 2009 review included pathogenic *E. coli, Campylobacter, Salmonella, Leptospira, Cryptosporidium*, and *Giardia.*

Pathogens not covered in this TSM are nonzoonotic pathogens, pathogens generally found in the environment, such as free-living protozoa, and pathogens with cold-blooded hosts, such as snails or copepods (U.S. EPA, 2009b). Additionally, some common zoonotic pathogens were excluded because they do not have a well-documented waterborne transmission but may be transmitted by other routes, such as food or soil.

Reviews of data on waterborne transmission of zoonotic pathogens have identified pathogens of primary concern based on their occurrence in water, abundance in animal feces, and persistence and ability to multiply in the environment (Rosen, 2000; Bicudo and Goyal, 2003; Goss and Richards, 2008; U.S. EPA, 2009a,b, 2013a,b). Based on these criteria, the protozoa *Cryptosporidium* and *Giardia*, the bacterial pathogens *Salmonella*, *Campylobacter,* and pathogenic *E. coli* (represented by *E. coli* O157:H7) are the primary pathogens of concern in livestock waste (Rosen, 2000; Bicudo and Goyal, 2003; Goss and Richards, 2008). Recreational water illnesses and their etiological agents of secondary concern include yersiniosis (caused by *Yersinia enterocolitica*), cercarial dermatitis (caused by schistosomes), shigellosis (caused by *Shigella* spp.), and leptospirosis (caused by *Leptospira* spp.) (Rosen, 2000; Levett, 2001; Bicudo and Goyal, 2003; Moyer and Degnan, 2006). There is little evidence of transmission of fecal-associated viruses of animal origin to humans, but some interspecies transmission of rotaviruses has been demonstrated experimentally (Rosen, 2000). Most viruses are host-specific, but a few are more general and are capable of infecting one or more species of host. Human and animal fecal wastes contain a variety of different kinds of viruses, and some animal viruses (e.g., hepatitis E) have the potential to infect humans (U.S. EPA, 2009b). However, there are considerable uncertainties about the waterborne transmission of zoonotic viruses to humans. Outbreaks of waterborne gastrointestinal illness are commonly attributed to human enteric viruses, such as norovirus (Hlavsa et al., 2015).

QMRA models estimate potential risk from exposure to specific pathogens. As part of the QMRA-based analyses discussed in this TSM, the EPA chose a short list of “reference” pathogens based on the key waterborne zoonotic pathogens found in feces and common human enteric viral pathogens. The specific pathogens, the justification for selecting the pathogens, and pathogen-specific parameters used in QMRA are presented along with information on the FIB, *E. coli* and enterococci, found in nonhuman fecal sources.

2.1.1 Reference and Index Pathogens

The U.S. Centers for Disease Control and Prevention (CDC) estimates that known pathogens account for an estimated 33 million illnesses each year in the United States, including 4.2 million due to bacteria, 2 million due to parasites, and 27 million due to viruses (Table 2-1) (Scallan et al., 2011a,b). Of those illnesses, 9.3 million were thought to be foodborne, leaving 24 million illnesses, some of which were due to waterborne exposures, including recreational water contact. Of the 24 million nonfoodborne illnesses per year, 21.5 million illnesses are associated with a viral etiology, with norovirus estimated to account for approximately 15.3 million of the nonfoodborne illnesses. There is an overlap between the few pathogens attributed to most nonfoodborne illnesses in the United States and the list of key feces-associated waterborne zoonotic pathogens the EPA identified (U.S. EPA, 2009b). Research has demonstrated that bacteria (*Campylobacter* spp., *E. coli* O157:H7, and *Salmonella enterica*) and protozoa (*Cryptosporidium* and *Giardia* spp.) can be zoonotic pathogens (Till et al., 2008; Ahmed et al., 2009; U.S. EPA, 2009b; Gabriel et al., 2010; McBride et al., 2013; Schmidt et al., 2013; Hlavsa et al., 2015; Goh et al., 2019; Korajkic et al., 2019). These five zoonotic pathogens, which appear on both the CDC’s and

Table 2-1. Estimated annual illnesses in the United States from known pathogens.[a,b]

Pathogen class	Pathogen	Total estimated annual cases	Percent foodborne illness	Number of foodborne illnesses	Number of nonfoodborne illnesses[c]
Bacterial	*Bacillus cereus*	63,411	99.98	63,400	11
	Botulism, foodborne	55	100	55	0
	Brucella spp.	1,679	50	839	840
	Campylobacter spp.	1,058,387	80	845,024	213,363
	Clostridium perfringens	966,120	99.98	965,958	162
	E. coli O157:H7	93,094	68	63,153	29,941
	E. coli, non-O157 STEC	138,063	82	112,752	25,311
	E. coli, enterotoxigenic	17,897	99.98	17,894	3
	E. coli, other diarrheagenic	39,739	30	11,982	27,757
	Listeria monocytogenes	1,607	99	1,591	16
	Salmonella enterica serotype Typhi	1,897	96	1,821	76
	Salmonella, nontyphoidal	1,095,079	94	1,027,561	67,518
	Shigella spp.	421,048	31	131,254	289,794
	Staphylococcus aureus foodborne	241,188	100	241,188	0
	Streptococcus, foodborne	11,219	99.98	11,217	2
	Vibrio cholerae, toxigenic	84	100	84	0
	V. vulnificus	203	47	96	107
	Vibrio, other	30,727	57	17,564	13,163
	Yersinia enterocolitica	108,490	90	97,656	10,834
	Subtotal	**4,289,987**		**3,611,089**	**678,898**
Parasitic	*Cryptosporidium* spp.	678,828	8	57,616	621,212
	Cyclospora cayetanensis	11,522	99	11,407	115
	Giardia intestinalis	1,121,864	7	76,840	1,045,024
	Toxoplasma gondii	173,415	50	86,686	86,729
	Trichinella spiralis	156	100	156	0
	Subtotal	**1,985,485**		**232,705**	**1,753080**
Viral	Norovirus	20,796,079	26	5,461,731	15,334,348
	Rotavirus	3,089,868	< 1	15,433	3,074,435
	Astrovirus	3,089,868	< 1	15,433	3,074,435
	Hepatitis A	21,041	7	1,566	19,475
	Subtotal	**26,996,856**		**5,494,163**	**21,502,693**
Total		**33,272,328**		**9,337,957**	**23,934,493**

Notes:
a. Source: Scallan et al. (2011a,b)
b. Shaded pathogens represent the standard list of reference pathogens the EPA discusses in this TSM.
c. These can include recreational waterborne illnesses.

the EPA's lists, plus the human enteric viruses norovirus and rotavirus, are highlighted in gray in Table 2-1. To this list, the EPA added human adenovirus, a nonenveloped virus resistant to common wastewater treatment (because it has been identified as an etiologic agent in untreated recreational water outbreaks), documented to occur in sewage year-round and measured in waters affected by human fecal contamination (Lee et al., 2014; Hlavsa et al., 2015; Rames et al., 2016; Goh et al., 2019; Graciaa et al., 2018). Together, these eight pathogens can be considered reference pathogens for evaluating waterborne illnesses in QMRA. The use of reference pathogens to represent the infectivity and the likely environmental fate and transport of each microbial group is a widely accepted practice in the field of QMRA (WHO, 2004; Roser et al., 2007; Soller et al., 2010b; Haas et al., 1999, 2014).

The QMRA reference pathogens included in the QMRA modeling in this TSM (Text Box 2-1) were selected for recreational waters because they:

1. Represent the three classes of pathogens of concern (bacteria, viruses, and protozoa),
2. Are found in a wide variety of sources,
3. Are transmitted via water exposure,
4. Are known to cause gastrointestinal illness,
5. Have associated peer-reviewed dose-response relationships in the literature, and
6. Account for more than 97% of nonfoodborne illnesses in the United States (Mead et al., 1999; Scallan et al., 2011a,b).

Text Box 2-1. Reference and Index Pathogens

Reference and index pathogens represent the infectivity and the fate and transport characteristics of the pathogens most likely to occur in waterbodies affected by warm-blooded animal (including human) fecal sources. The reference pathogens in this list are associated with a significant proportion of waterborne gastrointestinal illnesses. The index pathogens listed represent a broader group of pathogens that, as a group, are important. This set of reference and index pathogens is useful because it covers the major waterborne pathogens associated with nonfoodborne illness occurrence in the United States.

The reference pathogens include the following:

- Bacteria: *Campylobacter* spp., *Salmonella enterica*, and *E. coli* O157:H7 (as an index for the broader group of pathogenic *E. coli* and other gram-negative, facultative anaerobic, nonspore-forming bacteria that can cause diarrheal diseases such as *Shigella* spp.).
- Protozoa: Cryptosporidium and Giardia spp.
- Viruses: Norovirus (as an index for the broader group of enteric viruses[7]), rotavirus, adenovirus (as an index for viruses that are resistant to wastewater disinfection and have low attenuation rates in the environment).

[7] In this TSM, norovirus is considered an index pathogen because published dose-response functions have been shown to effectively model gastrointestinal illnesses reported in epidemiological studies (Soller et al., 2010a). Because norovirus occurrence in the population can vary over the year (with peaks during winter in the northern hemisphere) and the epidemiological data were collected during the summer, norovirus was unlikely to be the primary viral etiologic agent. Other enteric viruses, such as adenoviruses and enteroviruses, causing similar symptoms and resulting in quick-onset, usually self-resolving acute gastrointestinal illness (AGI) were likely present because the beaches characterized in the epidemiological studies were affected by nearby discharges of secondary treated and disinfected effluent and the symptomology of illness was consistent with a viral etiology. See Wade et al. (2006, 2008, 2010, 2022) for a discussion of the health effects reported during EPA's NEEAR epidemiological studies.

Of the eight reference pathogens described above, the CDC estimated the number of annual illnesses for seven of these pathogens (gray rows in Table 2-1). Adenovirus, norovirus, and *E. coli* O157:H7 are also considered index pathogens because they represent a broader class of pathogens that share similar infectivity or environmental fate and transport characteristics, can be waterborne, and have been included in published QMRAs (WHO, 2004; Pond, 2005; Roser et al., 2007; Soller et al., 2010a,b; Ferguson et al., 2008; Schoen et al., 2011; Hlavsa et al., 2015; Graciaa et al., 2018). For the purposes of this TSM, the index pathogens are considered a subset of the reference pathogens. Hereafter, these eight pathogens are referred to collectively as reference pathogens.

Adenoviruses are nonenveloped, double-stranded deoxyribonucleic acid (DNA) viruses that are very stable after chemical or physical agent exposure or adverse potential of hydrogen (pH) conditions, are stable and persistent with a wide distribution in a range of water matrices, found consistently in sewage, have high resistance to ultraviolet disinfection, detected in chlorinated wastewater, and occur in higher abundance compared to other enteric viruses (Pond, 2005; Rames et al., 2016). Adenovirus is not a reportable disease agent in the United States, so the health burden from adenovirus exposures in recreational waters is likely underestimated but is known as one of the leading causes of recreationally associated waterborne disease worldwide (Sinclair et al., 2009). Adenovirus is the most prevalent type of human enteric viruses in water and has been identified as the etiologic agent responsible for some outbreaks in untreated recreational water (Pond, 2005; Jiang, 2006; Hlavsa et al., 2015; Graciaa et al., 2018). There are available culture and gene-based assays to enumerate adenoviruses, which are easier to detect than ribonucleic acid (RNA) viruses (Rames et al., 2016; Hess et al., 2021). Because adenoviruses can replicate and achieve a high viral load in the gut before excretion in feces, contaminated water has been a documented source of exposure for adenoviruses, either through ingestion, inhalation, or direct contact with the eyes (Singh-Naz and Rodriguez, 1996; Glass, 2001; Kelly and Birch, 2004; U.S. EPA, 2009b). Environmental monitoring studies have documented the occurrence of adenovirus in surface waters (Choi and Jiang, 2005; U.S. EPA, 2009b; Marion et al., 2014). Marion et al. (2014) reported significant positive associations between adenovirus exposure and diarrhea and gastrointestinal illness in recreators.

Norovirus was selected as a reference pathogen because it is a leading cause of recreational waterborne illness including outbreaks of gastrointestinal illness (Scallan et al., 2011a,b; Matthews et al., 2012; Gibson, 2014, Eftim et al., 2017; Graciaa et al., 2018). Norovirus is also considered an index pathogen because it can represent the broader class of enteric viruses present in human fecal contamination (Soller et al., 2010a; McBride et al., 2013). In Soller et al. (2010a), the Teunis et al. (2008a) norovirus dose response effectively indexed the vast majority of illnesses reported in the EPA's NEEAR water study.

E. coli O157:H7 is an index for pathogenic *E. coli* because it is representative of other pathogenic *E. coli* and other gram-negative, facultative anaerobic, nonspore-forming bacteria (e.g., *Shigella* spp.); is highly infectious; has peer-reviewed dose-response relationships; and can exhibit more serious health outcomes, such as hemolytic urea syndrome (HUS). The EPA selected *E. coli* O157:H7 to ensure that these types of outcomes would be included, even though the reported number of cases per year is relatively low.

While users of this TSM are not limited to these reference pathogens, they were selected for recreational waters based on a thorough review of the scientific literature and their use in other QMRA and water quality studies. Users of this TSM should provide supporting documentation if other

reference pathogens are included in the QMRA or if the standard reference pathogens are not included. If the collected source tracking information demonstrates little to no human inputs to a waterbody, a decision not to include the viral reference pathogens (adenovirus, norovirus, rotavirus) could be made because they are associated with human fecal sources. If human sources are present but are not dominating the fecal loading, monitoring for one or more of these viruses could be used as additional information on the extent of human fecal loading to the water being studied. For example, adenoviruses are known to persist in environmental waters, are consistently found in wastewater throughout the year and can be detected with both culture- and gene-based methods (Rames et al., 2016). In mixed-source scenarios with low human inputs, recent advances made in methods for viral enumeration could be considered (Haramoto et al., 2018). Given this information, the selection of the reference pathogens should be based on the sources of contamination.

This section discusses the QMRA parameters related to the reference pathogens, including:

1. Occurrence of reference pathogens in feces and water.
    a. Abundance of pathogens in fecal material for humans, cattle, gulls, pigs, and sheep (Table 2-2).
    b. Prevalence of pathogens in animal feces (proportion of animals that are shedding) (Table 2-3).
    c. Environmental fate and transport of pathogens.
2. Infectivity and illness associated with reference pathogens.
    a. Human infection potential of pathogens in animal feces (Table 2-4).
    b. Dose-response relationships and parameters for dose-response models for each reference pathogen (Table 2-5).
    c. Probability of illness given infection for each reference pathogen (Table 2-6).

The QMRA framework presented in this TSM follows the infectious disease process of exposure, infection, and illness chronologically. For example, the first step is exposure to a pathogen through some behavioral activity. Once exposure occurs, the pathogen interacts with the host, which can lead to infection. However, not all exposures result in infection. Illness may or may not occur in an infected host. The human immune system can mediate the extent and severity of the exposure, infection, and illness process.

The process of environmental fate and transport of pathogens from excretion to entering surface waters is also presented to consider to what extent fate and transport parameters are included within the QMRA. A health-protective and parsimonious evaluation of illness risk can be associated with a direct deposition scenario where exposure occurs to fresh animal feces contaminating a recreational waterbody (Soller et al., 2010b). However, including some fate and transport information, if known, may provide information about fecal loading dynamics to surface waters, hazardous conditions, and evaluation of risk (Soller et al., 2015; Wu et al., 2020).

If cattle are identified as a contributing source of fecal loading to a waterbody, added considerations for planning and scoping are warranted. Soller et al. (2010b) conducted a QMRA evaluating the relative risks of gastrointestinal illness between human and animal fecal sources. They found that the potential risk from recreational exposure to direct deposition of fresh cattle feces in an untreated ambient recreational water body can be equivalent to the risk from treated human point source discharges at the same level of culturable enterococci. The fecal pathogen profiles differ between cattle and human fecal sources. Conversely, when using culturable *E. coli* to estimate the potential risk of illness, direct deposition of cattle feces was less risky than treated human discharges but similar to the risk posed by raw sewage at the same level of culturable *E. coli*. The difference in risk estimates can be affected by the relative levels of pathogens reported in fecal sources compared to levels of the FIB (see Table 2-7). Cattle excrete, on average, much more *E. coli* compared to enterococci. Furthermore, when considering certain fate and transport characteristics, such as land deposition and event-driven runoff on the potential risks from pastured cattle, the probability of gastrointestinal illness from recreational exposures to cattle feces can be reduced compared to the direct deposition scenario (WERF, 2011; Soller et al., 2015). Other QMRA-based analyses have reported the potential to overestimate health risks when microbial decay characteristics are not considered in the modeling (Wu et al., 2020). When best management practices (BMPs) prevent direct contact of cattle with water and cattle feces are well managed to prevent mass loading to water, the risk of gastrointestinal illness from cattle feces can be reduced (U.S. EPA, 2010c; Soller et al., 2015; Wu et al., 2020).

2.1.1.1 Prevalence and Abundance of Pathogens in Animal Feces

The observed ranges of pathogens in the feces of different animals and humans (Table 2-2) have been reported in the scientific literature (Schoen and Ashbolt, 2010; Soller et al., 2010b, 2018; U.S. EPA, 2010c; Boehm and Soller, 2020). For this TSM, the criteria used to select pathogen prevalence and abundance data and numerical ranges from the literature for use in QMRA were:

1. Preferred data were from large-scale studies of long duration.
2. Conducted in the United States.
3. Based on individual (not composite) samples.

The values in Table 2-2 can be used to conduct QMRA as outlined in Section 3, Step 3. The ranges in Table 2-2 can be included in the QMRA modeling as a distribution between the high and low values, with each value equally likely (i.e., uniformly distributed). Table 2-2 includes human (raw and treated sewage), and animal (cattle, chickens, gulls, and pigs) sources. *E. coli* O157:H7 has been found in cattle, human, and pig feces but has not been reported in chicken or gull feces. *Campylobacter* and *Salmonella* were found in human, pig, chicken, and gull feces. *Cryptosporidium* and *Giardia* were found in human and pig feces but not reported in chicken or gull feces. Norovirus was found only in human feces. The six pathogens, *E. coli* O157:H7, *Campylobacter*, *Salmonella, Cryptosporidium, Giardia,* and norovirus, were all detected in raw sewage (Stampi et al., 1993; Wallis et al., 1996; Grant et al., 1996; Jiménez-cisneros et al., 2001; Lemarchand and Lebaron, 2003; Metcalf and Eddy, 2003; Rose et al.,

Table 2-2. Density of reference pathogens in human and selected animal fecal waste (adapted from Soller et al. [2010b, 2018]).

Fecal source			Density[a] of: *E. coli* O157:H7 min[b]	max[c]	*Campylobacter* spp. min	max	*Salmonella* spp. min	max	*Cryptosporidium* spp. min	max	*Giardia lamblia* min	max	Norovirus
Human	Raw sewage	Range[d]	ND[e]	3.3	2.95	4.6	0.48	7.38	−0.52	4.38	0.51	4.95	Log mean = 4.7 (Log StDev = 1.5)
Human	Secondary treated and chlorinated effluent	Range[d]	ND		ND		ND		−1.0	1.5	−1.0	2.1	Attenuation range $(\log_{10})$[f] 2.2 to 3.0
Animal	Cattle	Range[d]	3.1[g]	8.4	1.2	7.3	3.0[h]	5.8	2.3	3.9	0	4.9	NA[i]
		Basis[j]	W		W		W		W		W		
		Type[k]	C		D		C		C		D		
	Chicken	Range[d]	NR		2.8	6.5	−1.0	4.5	ND		NR		NA
		Basis[j]			W		D						
		Type[k]			D		D[l]						
		Manure[m]			F		F						
	Gulls	Range[d]	NR[n]		3.3	6.0	2.3	9.0	NR		NR		NA
	Pigs[p]	Range[d]	ND	7.0	2.0	5.7	2.8[g]	4.9	1.7[g]	3.6	0	6.8[o]	NA
		Type[k]	D		D		C		C		D		

Notes:

a. Values represent exponent in the power of 10 exponential (e.g., n = 10n)
b. Denotes minimum observed value
c. Denotes maximum observed value
d. For raw sewage and secondary chlorinated effluent, units of minimum and maximum observations are CFU per liter (L) or oocysts per L or cysts per L; for livestock wastes, units are CFU per gram (g) or oocysts per g or cysts per g; norovirus is in units of genomes per L.
e. Not detected (ND).
f. Removal range (rather than range of density). Attenuation in treatment (in log10 units) is assumed to be uniformly distributed.
g. GM (minimum observed density not reported).
h. Low end of range of values "typically measured in cattle manure." Actual minimum not presented.
i. Not applicable (NA), not usually considered to be present in this source.
j. Basis refers to weight basis for manure. D denotes dry weight and W denotes wet weight.
k. Sample type is either composite (C) or direct (D).
l. Samples were taken at random from the top of the litter pile. Because the droppings were fresh, they presumably came from a single bird.
m. Chicken manure type is litter (L) or fresh (F).
n. None reported (NR); no data were found in the literature to quantify densities in this source.
o. Estimated from data presented graphically.
p. All pig fecal abundances reported are for solid, fresh fecal samples (not slurries or treated manure).

2004; Garcia-Aljaro et al., 2004, 2005; Haramoto et al., 2006; Robertson et al., 2006; Katayama et al., 2008). In effluent from secondary treatment and chlorination, *E. coli* O157:H7, *Campylobacter*, and *Salmonella,* were not detected (ND) (Stampi et al., 1993; Lemarchand and Lebaron, 2003; Rose et al., 2004; Garcia-Aljaro et al., 2004; Haramoto et al., 2006; Whiley et al., 2013). *Campylobacter* and *Salmonella* were the only pathogens included for gulls in the QMRA model (Lévesque et al., 2000; Fogarty et al., 2003; Haack et al., 2003; Schoen and Ashbolt, 2010). For pigs, *E. coli* O157:H7, *Campylobacter*, *Salmonella, Cryptosporidium,* and *Giardia* were included in the QMRA model (Weijtens et al., 1999; Cornick and Helgerson, 2004; Hutchison et al., 2004; Maddox-Hyttel et al., 2006; Peu et al., 2006). For chickens, *Campylobacter* and *Salmonella* were the only pathogens included in the QMRA model (Kraft et al., 1969; Terzich et al., 2000; Cox et al., 2002; Hutchison et al., 2004; Doane et al., 2007; Brooks et al., 2009). For cattle, fecal densities of *E. coli* O157:H7, *Campylobacter*, *Salmonella, Cryptosporidium,* and *Giardia* were found in the literature (Hutchison et al., 2004; Atwill et al., 2006; Moriarty et al., 2008).

Note that the units for the different enumeration methods differ. For example, culture-based methods are usually in CFU or most probable number (MPN), whereas molecular methods can be in genome copies, cell equivalents, or PCR units. Transparent risk assessment documentation includes a discussion of the units for the various parameters, specifically where occurrence data do not have the same units as the dose-response relationship. In some cases, a conversion factor for units might be necessary (McBride et al., 2013).

The proportion of animals shedding pathogens, also known as prevalence, can differ among animals, with cattle producing a high proportion of *E. coli* O157:H7 and *Cryptosporidium* loading, poultry and dairy cattle contributing significantly to *Campylobacter* loading, and swine and poultry contributing to loadings of *Salmonella*. Manure handling methods vary widely among U.S. farms, with some directly depositing manure on pastures as solids or slurries (Bicudo and Goyal, 2003; U.S. EPA, 2013a). Manures might also be stored for variable amounts of time before application, which can differentially affect the attenuation of pathogens and the viability of bacterial indicators (Sobsey et al., 2006; Soller, 2015). The exposure modeling includes pathogen shedding (Table 2-3). The prevalence columns in Table 2-3 represent the percentage of animals shedding within a herd or flock at any given time. The average of the minimum and maximum values was calculated as a point estimate and included in the dose estimation calculations. For example, for *Campylobacter* in pigs, 72% was used (0.72 probability). For gulls, of the zoonotic pathogens, only *Campylobacter* and *Salmonella* were reported (Lévesque et al., 2000). *Campylobacter* and *Salmonella* in gulls had a lower bound based on levels detected by quantitative polymerase chain reaction (qPCR) (Lu et al., 2011; Rodríguez et al., 2012) and an upper bound of 100% based on composite samples (Lévesque et al., 2000). Of the five zoonotic pathogens listed, all but *Campylobacter* had less than 50% prevalence in pigs (Heitman et al., 2002; Cornick and Helgerson, 2004; Dorner et al., 2004; Hutchison et al., 2004; Xiao et al., 2006; Dorr et al., 2009). In chickens, *Campylobacter* and *Salmonella* had higher than 50% prevalence (Ley et al., 1988; Chapman et al., 1997; Byrd, 1998; Martin et al., 1998; Cox et al., 2002; El-Shibiny et al., 2005; Doane et al., 2007).

Table 2-3. Literature-reported prevalence (%) of reference pathogens in cattle, chicken, gulls, and pigs (adapted from Soller et al. [2010b]).

Source	*E. coli* O157:H7		*Campylobacter* spp.		*Salmonella enterica*		*Cryptosporidium* spp.		*Giardia lamblia*	
	min[a]	max[b]	min	max	min	max	min	max	min	max
Cattle	9.7	28	5	38	5	18	0.6	23	0.2	37
Chickens	0	0	57	69	0	95	6[c]	27[c]	NR[d]	
Gulls[e]	NR		54	100	75	100	NR		NR	
Pigs	0.1	12	46	98	7.9	15	0	45	3.3	18

Notes:

a. Denotes minimum observed value.

b. Denotes maximum observed value.

c. The *Cryptosporidium* strains in chickens are not infectious to humans (Ley et al., 1988). In Tables 2-2 and 2-4 (below), human-infectious *Cryptosporidium* was considered undetected in chickens.

d. NR, no data were found in the literature to quantify densities in this source.

e. For gulls, fecal prevalence and abundance data were based on observations from composite samples. All samples yielded campylobacters and salmonellae, so an upper bound of 100% prevalence was used. No samples yielded *E. coli* O157:H7, *Cryptosporidium*, or *Giardia*.

The relative fraction of human-infectious strains of each reference pathogen in the nonhuman sources can be variable; therefore, assigning an infectious fraction value for any particular source has an associated uncertainty. Insufficient data were available to assign consistent quantitative values for this parameter. Thus, a qualitative assessment of this parameter was used where categorical values of low (L), medium (M), or high (H) were assigned to each pathogen for each nonhuman source (Table 2-4). The qualitative potential for human infection was based on the known proportion of human-infectious species/strains/serotypes/isolates in animal feces. For human sources, the relative fraction of human-infectious strains is assumed to be 1.0 because the indicator and pathogen data are from sewage and not individual fecal samples and, therefore, already account for the fraction of humans who shed at any given time. For cattle, chickens, gulls, and pigs, point estimates were used to characterize the fractions of human-infectious strains based on the midpoint of the ranges of 0%–33% for low, 33%–66% for medium, and 67%–100% for high. Therefore, low is 16.5%, medium is 50%, and high is 83.3%.

In gull feces, *Campylobacter* and *Salmonella* were considered to have a high fraction of human infectious strains (Lévesque et al., 2000). In pig feces, *Cryptosporidium* was considered to have a low fraction of human infectious strains, *Salmonella* a medium fraction, and *E. coli* O157:H7, *Campylobacter*, and *Giardia* a high fraction (Heitman et al., 2002; Cornick and Helgerson, 2004; Dorner et al., 2004; Hutchison et al., 2004; Xiao et al., 2006; Dorr et al., 2009). In chicken feces, *Campylobacter* and *Salmonella* were considered to have a medium fraction of human infectious strains (Chapman et al., 1997; Byrd, 1998; Ley et al., 1988; Martin et al., 1998; Cox et al., 2002; El-Shibiny et al., 2005; Doane et al., 2007). In cattle feces, *E. coli* O157:H7, *Campylobacter*, *Cryptosporidium*, and *Giardia* were considered to have a high fraction of human infectious strains and *Salmonella* was considered to have a medium fraction of human infectious strains (Wesley et al., 2000; Fayer et al., 2000; Wade et al., 2000; Hoar et al., 2001; Sturdee et al., 2003; Hutchison et al., 2004; Fossler et al., 2005; Atwill et al., 2006; Berry et al., 2007).

Table 2-4. Fraction of human infectious strains of reference pathogens in cattle, chickens, gulls, and pigs (adapted from Soller et al. [2010b]).

Source	*E. coli* O157:H7	*Campylobacter* spp.	*Salmonella enterica*	*Cryptosporidium* spp.	*Giardia lamblia*
Cattle	H	H	M	H	H
Chickens	NR[a]	M	M	NR	NR
Gulls	NR	L	L	NR	NR
Pigs	H	H	M	L	H

Notes: Potential for human infection (H = high, M = medium, L = low) was based on the prevalence of known human-infectious species/strains/serotypes/ isolates in animal feces.
a. NR; no data were found in the literature to quantify densities in this source.

2.1.1.2 Environmental Fate and Transport

Upon excretion, enteric pathogens are exposed to very different conditions on land or in water compared to the gastrointestinal tracts of animals or humans in which they previously resided. Numerous physicochemical (abiotic) and biotic factors can affect the viability and infectivity of these pathogens, and thus the magnitude of potential health risks, as they move from the point of excretion to the point of exposure (Bradford et al., 2013; Ahmed et al., 2018; Korajkic et al., 2018). Pathogen growth, persistence, and attenuation vary between pathogens, between environmental media, and with other conditions such as moisture content (or water activity), sunlight, temperature, pH, humidity, salinity, predation, and organic matter (Gale, 2005; Englehardt et al., 2009; U.S. EPA, 2010c: Annex 3; Ahmed et al., 2018). Zoonotic pathogens may survive in the environment for days to months, depending on the extant conditions, but survival rates for the specific pathogens of concern may not be well-defined for all possible conditions (Roberts et al., 2016). Uncertainty in the estimates of pathogen loads can be large, particularly for pathogens like *Cryptosporidium* spp. that can be highly infectious and for pathogens with frequent nondetection. These variability and uncertainty issues around model parameters have been characterized in previous QMRAs (Signor and Ashbolt, 2006; Petterson et al., 2007, 2009).

Atwill et al. (2002) describe the steps in the pathway followed by enteric microorganisms from fecal material to a specified downslope location (in this case, a receiving stream) as follows:

1. Rainfall of sufficient intensity erodes the top layer of the fecal material, releasing pathogenic organisms onto the wetted soil surface;
2. Rainfall intensity reaches infiltration capacity, and pathogenic organisms are carried downslope via sheet flow, preferential rill flow, or exfiltration in variable source areas;
3. Pathogens are transported downslope or infiltrate into the subsurface; and
4. Vegetative buffer strips intercept flows laden with pathogenic organisms and enhance infiltration.

Ferguson et al. (2003) divided processes governing the relationship between watershed pathogens and densities in surface waters into those most important in organism inactivation (water/osmotic potential, temperature, sunlight, pH, and inorganic and organic nutrients) and those most important in transport (adsorption/desorption effects, hydrological movement, and mechanical or biological

movement). Figure 2-2 illustrates the various factors affecting animal waste as it moves from the point of excretion to receiving waters via multiple pathways. The transport of pathogens and fecal indicator organisms in the waste is influenced both by the pathways for feces entering waterbodies and by additional factors that impact survival and attenuation of the microbes; each process and factor have associated variability.

Avian fecal contamination can also reach recreational waters by multiple routes (Figure 2-3), including:

- Direct deposition as feces into the water column.
- Direct deposition via mechanical transfer (e.g., carried to receiving water on the legs of birds wading in sewage or sewage-impacted water) into the water column.
- Resuspension of fecal-associated pathogens from sediment.
- Precipitation-induced runoff of pathogens (either deposited or progeny of deposited organisms) from soil, vegetation, or impervious areas near the recreation area.
- Advection of bird-origin feces from stocks (e.g., in wetlands hydraulically connected to recreation site waters during high tides or flooding, as noted by He et al., 2007).

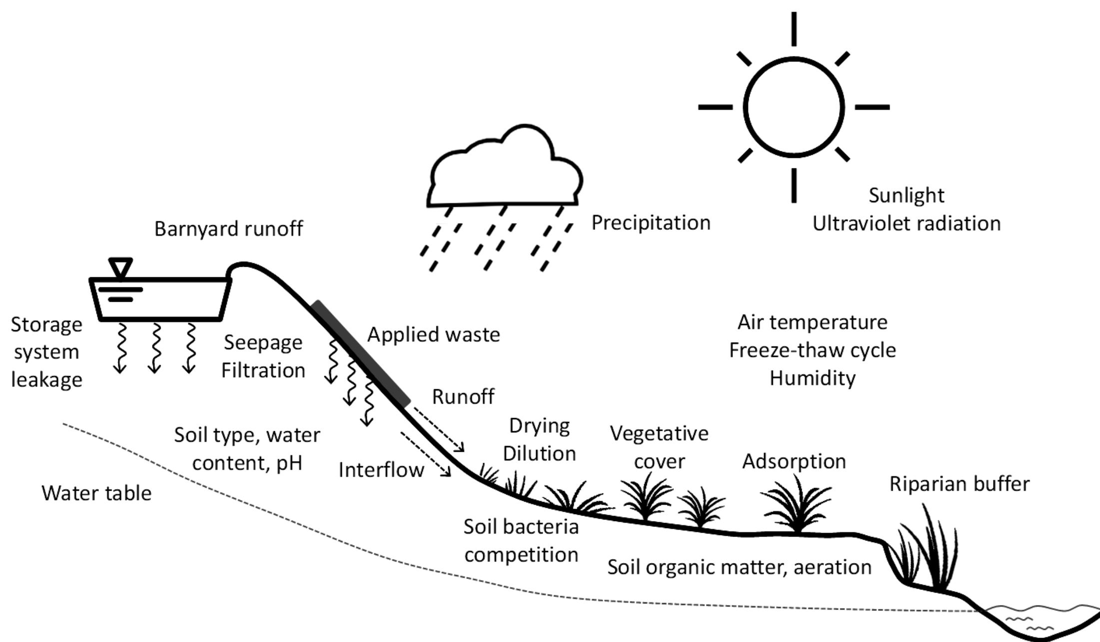

Figure 2-2. Factors affecting the viability of pathogens and indicators from deposition to surface waters (adapted from Rosen [2000]).

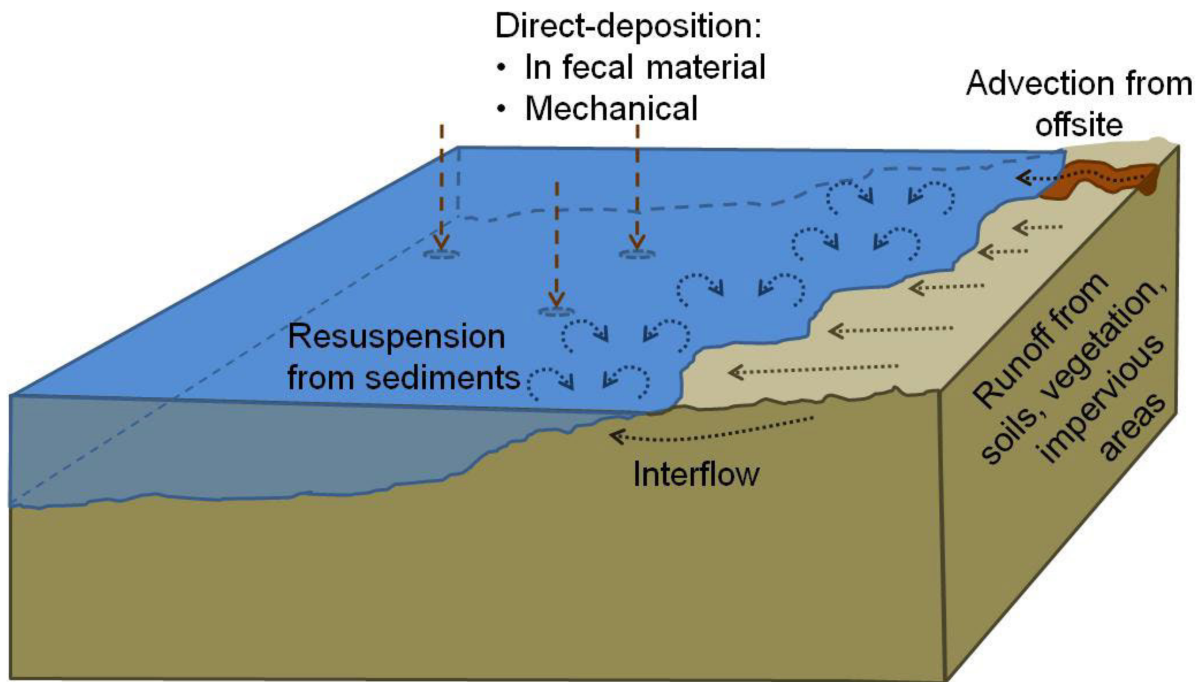

Figure 2-3. Routes of avian fecal contamination at a recreational freshwater beach.

Microbial runoff from soil is affected by numerous factors. Rather than characterize each factor separately, the EPA conducted field experiments for a defined typical rain event (Appendix E in U.S. EPA, 2010c). Plots receiving cattle, chicken, and swine manure applications at typical agronomic rates were subjected to an intense (< 100-year return period) rain event and the plot-scale runoff was monitored for reference pathogens and FIB. The calculated mobilization fractions for the zoonotic reference pathogen and FIB were subsequently incorporated into a QMRA, and risks from recreational exposure to the undiluted runoff were characterized (Soller et al., 2015).

Selection of pathogen enumeration methods can also affect fate and transport and potentially influence risk characterization (Rogers et al., 2011; Eichmiller et al., 2014). The use of culture-dependent or culture-independent (e.g., gene-based) enumeration methods can provide different information on the environmental decay of a pathogen and may or may not provide information characterizing the infectivity of the target. For example, the ultraviolet (UV) light component of sunlight typically increases decay rates, but the magnitude of this effect is influenced by other environmental conditions and the methodology used to measure the target (Korajkic et al., 2018). UV exposure can attenuate viable cells, which affects culture-based enumerations more readily than the corresponding nucleic acids. In low-nutrient, high-stress environments, many pathogens can enter a “viable, but non-culturable” (VBNC) state (Roberts et al., 2016). VBNC pathogens that are not detectable using culture-based methods may be detected by a gene-based enumeration method, such as qPCR.

The EPA used a health-protective approach in its QMRA framework that minimizes the variability associated with these fate and transport processes. In the exposure scenario to estimate human health effects, the EPA assumes direct deposition of feces to a waterbody or indirect deposition characterized as exposure to zoonotic pathogens in runoff because the levels of pathogens in these scenarios are the highest and would result in health-protective estimates. Although these assumptions might overestimate the potential for illness associated with inputs from a given source of fecal

contamination, the estimates generated with this approach minimize the uncertainties accompanying the multiple microbial fate and transport and loading parameters. Sunger et al. (2019) estimated illness risk to swimmers exposed to treated effluent-affected waters and incorporated the literature-reported microbial decay rates in freshwaters. Additional fate parameters can be included in the QMRA model discussed in this TSM, but the EPA's approach is more straightforward to conduct and allows for consistent application of the framework across many sites with varying characteristics. Illness probability estimates using this QMRA framework represent an upper bound on the potential for human health effects from recreational exposure to a nonhuman source of fecal contamination.

2.1.1.3 Infectivity and Illness

This subsection presents summary information on the reference pathogens' ability to cause infection given exposure and the probability of illness given infection. Recreators' exposure to fecal contamination in ambient waters can result in illness and, therefore, be a public health concern. Infectivity refers to the ability of a pathogen to cause or transmit an infection (U.S. EPA and USDA, 2012). The probability of illness in an exposed individual results from an interaction between the presence of the pathogen in the environment, the virulence and pathogenicity of the pathogen, and the immune status of the host. Figure 2-4 displays the epidemiological triangle, which is a model conceptualizing the agent-environment-host interaction as a means to consider parameters that influence public health outcomes (CDC, 2011; U.S. EPA, 2014c). Illness occurs when a pathogen capable of causing disease infects a host that is vulnerable to infection (Gulis and Fujino, 2015).

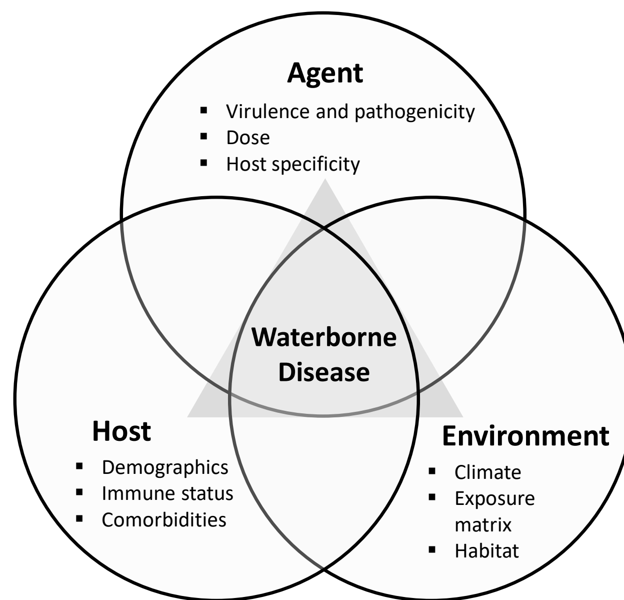

Figure 2-4. Epidemiologic triangle (adapted from CDC [2011]).

Recreational epidemiological studies (Cabelli, 1983; Dufour, 1984; Kay et al., 1994; Prüss, 1998; Wade et al., 2003, 2006, 2008, 2010; Fewtrell and Kay, 2015) have evaluated health endpoints among recreators with self-reported symptoms following exposure to waters affected by fecal contamination and include:

1. "Gastrointestinal illness," typically defined by any of the following within 10–12 days after swimming: (a) diarrhea (three or more loose stools in a 24-hour period), (b) vomiting, (c) nausea and stomachache, or (d) nausea or stomachache and impact on daily activity;

2. "Upper respiratory illness," which was defined as any two of the following: sore throat, cough, runny nose, cold, or fever;

3. "Rash," which was defined as a rash or itchy skin;

4. "Eye ailments," which were defined as either an eye infection or a watery eye;

5. "Earache," which was defined as ear pain, ear infection, or runny ears; or

6. "Infected cut," which was defined as a cut or wound that became infected.

At a marine subtropical beach with nonpoint source inputs, Sinigalliano et al. (2010) reported an increase in gastrointestinal, respiratory, and skin illnesses among bathers versus nonbathers. Colford et al. (2007) characterized health outcomes among recreators at Mission Bay, California, a nonpoint source-affected beach. They found an increase in diarrhea and rashes among those having any water contact and increased reports of cramps and eye ailments in those who swallowed water. Calderon et al. (1991) studied a freshwater recreation site with nonpoint source animal fecal inputs and no known human fecal inputs; they reported an increase in gastrointestinal illness among bathers, although increases in illness were associated with increasing bather density. Recreational water epidemiological studies generally do not directly characterize the etiologic agents of the reported illness in the waters studied. Some of the health endpoints listed above can be caused by one or more pathogens, and, in some cases, multiple endpoints can be attributed to a single pathogen.

Published QMRA studies characterizing different fecal contamination sources and dynamics have demonstrated that the nature (i.e., type of source and how the source affects surface waters) and magnitude of the fecal source(s) affecting a waterbody directly influence the probability of illness in recreators exposed to the contamination (Table 1-2). The etiologic agents of concern differ for human and nonhuman sources of contamination (Schoen and Ashbolt, 2010; Soller et al., 2010a,b; McBride et al., 2013; Sunger et al., 2019). Using QMRA, the EPA evaluated direct and indirect deposition from human and animal fecal contamination and mixtures of human and animal fecal contamination sources for the probability of illness, the potential etiologic drivers of illness, and estimating equivalently protective FIB levels (compared to the EPA's 2012 RWQC) for waters predominantly affected by nonhuman fecal sources (U.S. EPA, 2010a; Soller et al., 2014, 2015). The case study examples described in this document illustrate that the potential risk associated with a specific level of FIB from nonhuman fecal contamination can be significantly lower compared to human sources (Soller et al., 2010b, 2015). The risk differential is influenced by the difference in pathogen profiles present in different sources, the loading of that source to a waterbody and the relative level of culturable FIB among fecal sources. Other QMRA examples from the literature demonstrate the source with the highest pathogenicity, not necessarily the source(s) of greatest fecal loading, influences the risk of adverse health effects (Schoen

et al., 2011; Soller et al., 2014). These examples help to illustrate that considering the risk from nonhuman fecal loading can allow for the development of alternative WQC.

The following subsections describe the reported health outcomes, dose-response relationships, and morbidity reported in the literature for each reference pathogen included in the QMRA framework discussed in this TSM document.

2.1.1.3.1 Hazard Identification for Reference Pathogens

The eight reference pathogens discussed in this document are agents that are known to cause waterborne illness and account for more than 97% of nonfoodborne illness in the United States (Mead et al., 1999; U.S. EPA, 2009b, 2010a; Scallan et al., 2011a,b). The CDC reported 140 recreational water-associated outbreaks caused by pathogen, toxin, or chemical exposure from 2000 to 2014 (Graciaa et al., 2018). Enteric pathogens accounted for 80 documented outbreaks of waterborne disease. Although waterborne disease outbreak surveillance information represents an underreporting of the actual potential health burden, the reference pathogens discussed in this document were reported to account for approximately 53% of all recreational water-associated outbreaks from 2000 to 2014, where an etiologic agent was determined (Graciaa et al., 2018).[8] *Salmonella* and rotavirus were not identified as a known etiologic agent in these outbreaks.

Vanden Esschert et al. (2020) report on illness outbreaks associated with untreated recreational water in California, Maine, and Minnesota during 2018 and 2019. These outbreaks were voluntary entries in the CDC's National Outbreak Reporting System (NORS). Summary information provided on 31 states for the 2009–2019 period included 88 outbreaks with confirmed etiologies: norovirus (19 [22%] outbreaks; 1,858 cases); Shiga toxin-producing *E. coli* (STEC) (19 [22%] outbreaks; 240 cases); *Cryptosporidium* (17 [19%] outbreaks; 237 cases); and *Shigella* (14 [16%] outbreaks; 713 cases) (Vanden Esschert et al., 2020). Among the reference pathogens discussed in this TSM, *E. coil* O157:H7 is the most commonly identified STEC in the United States. (Minnesota Department of Health, 2022).

In this section, information about infectivity and adverse health outcomes reported in the literature for each of the reference pathogens is summarized. Discussion of how and where the pathogen(s) initiates infection is helpful for understanding the context of how different exposures lead to infections. The mechanisms of infection are not covered in detail in this document because the multiple reference pathogens cover the three major groups of pathogens (i.e., viruses, bacteria, and protozoa) and their mechanisms differ between and among the groups. For all reference pathogens discussed in this document, gastrointestinal illness is a common acute adverse health outcome. The following summaries are not exhaustive but are provided to establish each pathogen's relevance to recreational water illness.

Adenovirus. Adenovirus infections have been identified as a causal agent of lower and upper respiratory disease, acute gastrointestinal illness (AGI), acute conjunctivitis, cystitis, pharyngoconjunctival fever, and diseases of the central nervous system and urinary tract (Enriquez and Thurston-Enriquez, 2006; Teixeiria et al., 2020). Adenoviral lower respiratory tract infections are infrequent, sporadic, and most commonly associated with adenovirus types 3, 5, and 7 (Murtagh et al., 1993; Mandell, 2000). Epidemic keratoconjunctivitis is associated with adenovirus serotypes 8, 19, and 37. Acute respiratory

[8] Graciaa et al. (2018) report outbreaks for *Campylobacter, E. coli, Shigella, Cryptosporidium, Giardia,* adenovirus, and norovirus. In this TSM, *E. coli* O157:H7 is an index pathogen for the broader group of toxin-producing *E. coli* and other gram-negative, facultative anaerobic, nonspore-forming bacteria capable of causing waterborne disease (e.g., *Shigella* sp.).

disease is most often associated with adenovirus types 4 and 7. Enteric adenoviruses 40 and 41 cause gastroenteritis, usually in children (Wilhelmi et al., 2003). Many adenovirus serotypes can multiply in the small intestine, but only types 40 and 41 have been strongly associated with gastroenteritis (Grimwood et al., 1995). Adenoviruses have a low infective dose with approximately 5 viral particles needed to cause disease in a susceptible individual (Teixeiria et al., 2020).

Children, infants, older adults, the immunocompromised, and other sensitive populations can be especially vulnerable to developing infections (Jiang, 2006). Infection is most often spread via direct contact with an infected individual, the fecal-oral route, aerosol inhalation, or recreational water activities (which can include both oral and inhalation exposure). Adenovirus exposure has been significantly associated with gastrointestinal illness in recreational waters (Marion et al., 2014). Although not thought to be the most common route of transmission, two drinking water outbreaks have been reported in Europe in which enteric adenoviruses were suspected as the cause of the reported acute gastroenteritis (Mena and Gerba, 2009). Adenoviruses in drinking water also might contribute to viral infections of unknown etiology (Ko et al., 2003).

Neurological sequelae from Reye's syndrome following infections by adenovirus have been reported (Daugherty and Heubi, 1985). For example, studies of Reye's syndrome survivors have shown sequelae ranging from severe psychomotor retardation to mild specific perceptual and language impairments, intelligence quotient (IQ) deficits, and chronic behavioral deficits that lasted from six to 18 months after infection (Davidson et al., 1978; Brunner et al., 1979; Shaywitz et al., 1982).

Norovirus. Noroviruses are highly contagious and transmitted primarily through the fecal-oral route—most commonly by ingesting fecally contaminated food or water or through person-to-person spread (Schwab and Hurst, 2006; CDC, 2023). Three of the six norovirus genotypes are infective to humans and are referred to as genogroups I, II, and IV. Genogroup II noroviruses are thought to be the predominant cause of infections (Teixeira et al., 2020). In temperate latitudes, norovirus is associated with "winter vomiting disease" (Hutson et al., 2004). Environmental and fomite[9] contamination can also be an important source of transmission and infection. Matthews et al. (2012) conducted a meta-analysis of outbreaks (n = 902) and found that the primary attack rate for food (50%) and water (38%) was higher than the attack rate for other exposures (e.g., person-to-person [27%], fomites [26%]). Waterborne outbreaks of norovirus disease in community settings are often caused by sewage contamination of wells and recreational water (CDC, 2023) and are widely considered to be the causative agents in many viral waterborne outbreaks of unknown etiology (Maunula et al., 2005; U.S. EPA, 2006c). Although pre-symptomatic viral shedding can occur, shedding usually begins with the onset of symptoms and can continue for up to two weeks after recovery from illness (CDC, 2023). The incubation period for norovirus-associated AGI in humans is usually between 24 hours and 48 hours (median in outbreaks: 33–36 hours), but cases can occur within as little as 12 hours of exposure.

Infection usually presents as acute-onset vomiting, watery nonbloody diarrhea with abdominal cramps, and nausea. A low-grade fever also can occur, although dehydration is the most common complication, especially among young children and older adults. Symptoms can usually last 2–4 days (Teixeira et al., 2020). Adverse health outcomes are higher among young children, older adults, and immunocompromised persons (Hutson et al., 2004). A human volunteer feeding study was conducted to determine the infectivity of norovirus in healthy adults to predict the likelihood of enteric infection following ingestion exposure of norovirus in water (Teunis et al., 2008a). Although asymptomatic

[9] A fomite is an inanimate object or substance that can transmit infectious organisms from one individual to another.

infection can occur in as many as 30% of infections, its role in calicivirus/norovirus transmission remains poorly understood.

Rotavirus. Rotavirus infection is the leading cause of acute severe gastroenteritis in young children and infants in the United States and worldwide (Abbaszadegan, 2006). Symptoms of rotavirus infection include diarrhea, fever, and vomiting, leading to dehydration—especially in young children and infants. Rotaviruses are excreted in very large quantities in the feces of infected persons with diarrhea (10^{11} per mL), so they are present in relatively high densities in wastewater and ambient water (Ansari et al., 1991). Rotavirus is highly contagious (Gerba et al., 1996). Infection is most often spread via the fecal-oral route, contact with feces or virus-contaminated objects, direct contact with an infected individual, or contaminated food or water. In 2008, the CDC reported that each year, rotavirus infections accounted for an estimated 55,000 to 70,000 hospitalizations in the United States (CDC, 2008). The CDC estimates that introducing a rotavirus vaccine in 2006 has prevented an estimated 40,000 to 50,000 hospitalizations annually among U.S. infants and young children (CDC, 2018). Since the introduction of vaccines against rotavirus in 2006, the number of cases in the United States has declined. Although not common, drinking-water-related rotavirus outbreaks have occurred in the United States (e.g., Hopkins et al., 1984, 1985). Rotaviruses have been detected in treated drinking water (Abbaszadegan et al., 1999) and nondisinfected well water (Borchardt et al., 2003). Rotaviruses in drinking water also can contribute to waterborne outbreaks of unknown etiology and unknown levels of endemic disease.

Campylobacter jejuni. *Campylobacter* sp., most notably *C. jejuni*, is a major zoonotic pathogen. The primary adverse health endpoint associated with *C. jejuni* infection is AGI, characterized by diarrhea, fever, and abdominal cramps (Butzler, 2004). The incubation period is typically two to five days but can extend up to 10 days. In about 50% of patients, diarrhea is preceded by a febrile period associated with malaise, myalgia, and abdominal pain; fresh blood can also appear in the stool by the third day. Most *C. jejuni* infections are sporadic in nature and are often associated with outbreaks where the source of pathogen is not determined (Hänninen et al., 2003). In drinking water outbreaks attributed to *C. jejuni* (e.g., Jones and Roworth, 1996; Holme, 2003), the drinking water source is usually shown to be fecally-contaminated by runoff of surface water after rain or by leakage of a sewage pipe close to the drinking water pipeline.

McBride et al. (2002) found that 60% of all samples collected from 25 freshwater recreational water sites in New Zealand over 15 months contained at least one species of *Campylobacter*. This finding led to an inference that 4% of campylobacteriosis cases in New Zealand were due to water contact recreation (McBride et al., 2002).

C. jejuni is increasingly recognized as a risk factor for Guillain-Barré syndrome, a common cause of neuromuscular dysfunction (Bunning et al., 1997) that occurs in one in 1,000 infections (Butzler, 2004). Guillain-Barré syndrome is the most serious complication of *Campylobacter* infection (Kaldor and Speed, 1984; Nachamkin et al., 2000; Yuki, 2001; Ang et al., 2002; Gilbert et al., 2004). Studies by McCarthy and Giesecke (2001) have shown that the risk of developing Guillain-Barré syndrome during the two months following a symptomatic episode of *C. jejuni* infection is approximately 100 times higher than the risk in the general population.

Reactive arthritis is a rare complication of infection (FDA, 2012). Arthritis typically occurs within two weeks after the onset of gastrointestinal illness, but onset time can range from four to 35 days (Anonymous, 1998). The condition is characterized by infection at a distant site, whereby joint

inflammation occurs without typical evidence of sepsis at the affected joint(s) (Shirtliff and Mader, 2002).

Reiter's syndrome is an aseptic arthritis (Kim et al., 2007) that is triggered by an infectious agent located outside the joint. It also has a strong association with human leukocyte antigen-B27. Pope et al. (2007) reviewed the epidemiological literature on *Campylobacter*-associated reactive arthritis and found that follow-up for long-term sequelae related to *Campylobacter* and Reiter's syndrome was largely unknown.

Immunoproliferative small intestinal disease is an infectious pathogen-associated occurrence of human lymphomas (Al-Saleem and Al-Mondhiry, 2005), and molecular and immunohistochemical studies have demonstrated an association with *C. jejuni* (Lecuit et al., 2004). This disease involves the proximal small intestine, resulting in malabsorption, diarrhea, and abdominal pain, as well as weight loss, intestinal obstructions, and abdominal masses (Gilinsky et al., 1987; Rambaud et al., 1990; Fine and Stone, 1999; Al-Saleem and Al-Mondhiry, 2005). Symptoms are often chronic, and patients can experience mild symptoms for five to 10 years before developing higher-grade illness or lymphoplasmacytic and immunoblastic lymphomas (Al-Saleem and Al-Mondhiry, 2005).

E. coli O157:H7. *E. coli* O157:H7 has been found worldwide in feces-contaminated surface water, groundwater, nonchlorinated or inadequately treated drinking water and swimming pools, soil and sediment, and a wide variety of foods (Muniesa et al., 2006). It has been documented to cause both endemic illness and outbreaks in humans through fecal-oral transmission, consumption of feces-contaminated food and water, and contact with infected persons and animals (Rangel et al., 2005). The clinical symptoms of infection vary from nonbloody diarrhea to bloody diarrhea, referred to as hemorrhagic colitis. Hemorrhagic colitis is a serious, life-threatening condition and can lead to hemolytic uremic syndrome, which results in renal damage and possibly death (Tarr, 1995).

Enterohemorrhagic *E. coli* infections can lead to long-term or permanent kidney damage and renal disease. Persons who develop chronic kidney failure could require lifelong dialysis or a kidney transplant. Garg et al. (2003) conducted a comprehensive review and meta-analysis of the current literature along with expert consultations to quantify the long-term renal prognosis of patients with diarrhea-associated hemolytic uremic syndrome. A higher severity of acute illness was strongly associated with a worse long-term prognosis. Studies with a higher proportion of patients with central nervous system symptoms (coma, seizures, or stroke), had a higher proportion of patients who died or developed permanent end-stage renal disease at follow-up. Death or end-stage renal disease occurs in about 12% of patients with diarrhea-associated hemolytic uremic syndrome, and 25% of survivors demonstrate long-term renal sequelae.

Pathogenic *E. coli* are also implicated in rheumatoid diseases (Locht and Krogfelt, 2002; Schiellerup et al., 2008), including reactive arthritis and Reiter's syndrome with similar pathologies as described above. Pathogenic *E. coli* are phylogenetically related to *Shigella,* and some strains can produce shiga toxin (Mølbak and Scheutz, 2004)

Salmonella enterica. Most *Salmonella enterica* infections can be traced to contaminated food products, although water-related outbreaks (including drinking water outbreaks), have been reported (Schuster et al., 2005; CDC, 2006; Covert and Meckes, 2006; Craun et al., 2006). The incubation period ranges from 18–48 hours after ingestion, and illness is usually characterized by acute, self-limiting AGI (although some infections can be severe), fever, and septicemia, lasting 2–5 five days (Covert, 1999).

A broad range of chronic sequelae has been reported as consequences of *Salmonella* infection, such as reactive arthritis, Reiter's syndrome, rheumatoid syndromes, pancreatitis, osteomyelitis, myocarditis, colitis, cholecystitis, and meningitis (Thomson et al., 1995; Dworkin et al., 2001; Motarjemi, 2002).

Cryptosporidium sp. *Cryptosporidium* is a zoonotic waterborne pathogen of global public health importance. The CDC estimates that ~700,000 cases of *Cryptosporidium* infections a year occur in the United States (Scallan et al., 2011a). Worldwide, the estimated number of annual cases of cryptosporidiosis exceeds several million, and infection has been documented in almost 100 countries and on every continent except Antarctica (Casemore et al., 1997; Fayer et al., 1997; Cacciò, 2005). *Cryptosporidium* is a well-known cause of opportunistic infections among acquired immune deficiency syndrome (AIDS) patients (Roy et al., 2004) and a common cause of outbreaks of gastrointestinal illness (CDC, 2008). Surveillance data indicate that infections are more common among immunosuppressed individuals and that 90% of cases are not involved in outbreaks (Dietz et al., 2000). Valid species of *Cryptosporidium* have been described in more than 155 mammalian species, more than 30 avian species, 57 reptilian species, one species of fish, and two amphibian species (O'Donoghue, 1995; Fayer, 2004).

Most clinical cases of cryptosporidiosis involve infection by *C. parvum* or *C. hominis*. The most common feature of cryptosporidiosis is profuse, watery diarrhea. Other clinical signs of infection include dehydration, fever, anorexia, weight loss, weakness, and progressive loss of condition (O'Donoghue, 1995). Recovery is usually spontaneous within one to two weeks of infection. Developmental stages of the parasite are often seen within the small intestine and occasionally elsewhere (stomach, colon, liver, lungs). In general, the development of cryptosporidiosis depends on the host's species, age, and immune status (Fayer et al., 1997). Younger persons with less developed or compromised immune systems are often more susceptible to severe infection than healthy adults (O'Donoghue, 1995).

Several human volunteer feeding studies have been conducted to determine the infectivity of *C. parvum* and *C. hominis* in healthy adults to predict the likelihood of enteric infection following exposure to contaminated drinking water (DuPont et al., 1995; Chappell et al., 1996; Okhuysen et al., 1999, 2002). A summary of the dose-response data for all six tested isolates has been published (U.S. EPA, 2005). The EPA has also conducted additional dose-response modeling on *Cryptosporidium* (Messner and Berger, 2016).

Giardia sp. *Giardia intestinalis* (also known as *G. lamblia* and *G. duodenalis*) is a zoonotic waterborne pathogen of global public health concern. It is the most common intestinal parasite identified by public health laboratories in the United States (Rose et al., 1991; Mead et al., 1999). CDC estimates that approximately 1.2 million to 2 million illnesses occur annually in the United States due to *Giardia* (Mead et al., 1999; Scallan et al., 2011a). Of the U.S. general population, 30% has seropositivity for *Giardia* (indicates current or past infection), and 7% of small children are asymptomatically infected with *Giardia* (Frost and Craun, 1998). More than 100 waterborne giardiasis outbreaks have been reported worldwide from the beginning of the previous century to 2004 (Plutzer et al., 2010). *Giardia* also infects various domestic and wild mammals (e.g., cats, dogs, cattle, deer, beavers) (Thompson, 2000; Ballweber et al., 2010).

High-risk groups for giardiasis include infants and young children, travelers to developing countries, the immunocompromised, and persons who consume untreated water from lakes, streams, and shallow wells (U.S. EPA 1998a; CDC, 2008). A wide spectrum of symptoms is associated with giardiasis, from asymptomatic infection and acute self-limiting AGI to persistent chronic diarrhea, which might fail to respond to treatment. Asymptomatic infection is very common (50%–75% of infected persons are symptomatic) (Mintz et al., 1993; U.S. EPA 1998a). Symptoms of giardiasis include diarrhea, abdominal cramps, bloating, weight loss, and malabsorption (Rodriguez-Hernandez et al., 1996; Hellard et al., 2000; Thompson, 2000). Case reports also indicate that giardiasis might be associated with developing reactive arthritis (Tupchong et al., 1999). *Giardia* infection is frequently self-limited, but immunocompromised persons can have more serious and prolonged infection (Benenson, 1995). Hospitalizations and deaths due to giardiasis are relatively rare; CDC estimates that giardiasis causes approximately 10–30 deaths and 3,500–5,000 hospitalizations annually in the United States (Mead et al., 1999; Scallan et al., 2011a).

In some patients, symptoms last for only three or four days; in others, the symptoms last for months. Usually, patients commonly resolve their infections spontaneously, with acute disease lasting from one to four weeks (Smith and Wolfe, 1980). In some patients, however, the acute stage can persist for months (Wolfe, 1990). The period of communicability lasts for the entire duration of infection, but the shedding of cysts can be intermittent (Benenson, 1995).

Clinical data suggest that *Giardia* cysts are highly infective for humans (Rendtorff, 1954a,b; Rose et al., 1991; Teunis et al., 1996).

2.1.1.3.2 Zoonotic Infectivity Potential

Upon exposure to a pathogen, there is some probability of an infection being initiated. Infection is influenced by the pathogenicity or virulence of the pathogen and the susceptibility of the host. Mathematically, the probability of infection is described by a “dose response” function. Dose-response relationships for the reference pathogens presented in Table 2-5 were identified from the peer-reviewed literature and are for an infection endpoint. Definitions of infection most often used in dose-response models were seroconversion and shedding of pathogens in feces. The definition of reference pathogen illness also varied but was usually related to the incidence of diarrhea or vomiting. Narrative descriptions of the justification for the selected values follow.

Adenovirus. Teunis et al. (2016) provided a two-level dose-response model describing the distributions of infectivity and pathogenicity in various challenge studies of adenovirus, incorporating differences in inoculation route as shifts in average infectivity and pathogenicity. The combined challenge studies included a total of 91 subjects challenged in 23 dose groups. The infection dose-response relationship is an exact beta-Poisson (aka hypergeometric) relationship, and the conditional probability of illness given infection is dose-dependent. The hierarchical model was implemented in a Bayesian framework, the result of which was used to make predictions for the infectivity of adenovirus specific to any of the four studied inoculation methods (ingestion, inhalation, intranasal, and intraocular). The mean infection dose-response parameter estimates for oral exposures are $\alpha = 5.11$, and $\beta = 2.8$.

Table 2-5. Literature-reported dose-response relationships for reference pathogens.

Reference pathogen	Distributional form[a]	Parameter of distribution[b]	Parameter values	Reference
Rotavirus	Beta-Poisson	α	0.26	Regli et al., 1991
		β	0.42	
Norovirus	Hypergeometric[c]	α	0.393	Teunis et al., 2020
		β	0.767	
		W	mean = −0.608 variance = 1.79 covariance = −1.03	
		z	mean = 0.194 variance = 2.54 covariance = −1.03	
Adenovirus	Hypergeometric[d]	α	5.11	Teunis et al., 2016
		β	2.8	
Cryptosporidium spp.	Exponential	r	0.09	U.S. EPA, 2006a
Giardia lamblia	Exponential	r	0.0199	Rose and Gerba, 1991
Campylobacter jejuni	Hypergeometric[c]	α	0.44	Teunis et al., 2018
		β	0.51	
		W	mean = −0.177 variance = 1.303 covariance = −0.041	
		z	mean = 0.054 variance = 1.070 covariance = −0.041	
Campylobacter jejuni	Beta-Poisson	α	0.145	Medema et al., 1996
		β	7.59	
E. coli O157:H7	Beta-Poisson	α	0.248	Teunis et al., 2008b
		β	48.8	
Salmonella enterica	Beta-Poisson	α	0.3126	Haas et al., 1999; Fazil, 1996
		β	2884	

Notes:

a. Endpoints are infection. Dose-response model equations:

Exponential (Rose et al., 1991): $P_{infect}(dose; r) = 1 - e^{-r \cdot dose}$

Beta-Poisson (Regli et al., 1991; Rose and Gerba, 1991): $P_{infect}(dose; \alpha, \beta) = 1 - \left(1 + \frac{dose}{\beta}\right)^{-\alpha}$

Hypergeometric (Teunis et al., 2008a): $P_{infect}(dose; \alpha, \beta) = 1 - {}_1F_1(\alpha, \alpha + \beta, -dose)$

The ${}_1F_1$ function represents the confluent hypergeometric function that does not have a close form but is approximated by the beta-Poisson distribution when α « β and β » 1.

b. The parameters listed in this column correspond to the dose-response model (distributional form) listed for the pathogen.

c. Beta-Poisson model with the confluent hypergeometric function can use point estimates (α and β) or distributional estimates of α and β derived from the intermediate parameters *w* and *z*. The *w* and *z* distributions are defined by their mean, variance, and covariance.

d. Teunis et al. (2016) only reported point estimates for α and β for adenovirus.

Norovirus. Teunis et al. (2020) combined data from volunteer challenge studies and outbreaks in a multilevel dose-response framework that built on earlier Bayesian modeling of norovirus dose response (Teunis et al., 2008a). Infectivity and pathogenicity were analyzed by secretor status as a host factor (Se+ or Se−) and genogroup as a pathogen factor (Genogroup I or Genogroup II). Nonsecretors (Se−) are resistant to several norovirus genogroups (Nordgren and Svensson, 2019). The values selected in Table 2-5 correspond to the Se+ and Genogroup I modeling parameters provided by Teunis et al. (2020). The EPA selected this scenario because, in Se+ subjects, Genogroup I viruses appear slightly more infectious than Genogroup II viruses (Teunis et al., 2020). The Teunis et al. (2020) meta-analysis confirms the high infectivity of norovirus, with an estimated mean infection risk of 0.28 when exposed to one genome copy (qPCR unit) of Genogroup I norovirus, and 0.076 for one genome copy of Genogroup II norovirus, both in Se+ subjects.

Rotavirus. The rotavirus dose-response model is commonly used in risk assessment and is well accepted. The model was developed using data from human feeding studies (Ward et al., 1986a,b). Volunteers in the study were adult males 18–45 years old. Overall, the ratio of ill-to-infected individuals was 0.67, and the progression of infection to illness did not appear to be dose-dependent. Rose and Gerba (1991) used these data and reported parameter values of $\alpha = 0.232$ and $\beta = 0.247$. Haas et al. (1993) reported a beta-Poisson dose-response relationship for rotavirus infectivity with parameter values of $\alpha = 0.2531$ and $\beta = 0.4265$. Regli et al. (1991) published the widely accepted dose-response parameter values for rotavirus of $\alpha = 0.26$ and $\beta = 0.42$ (Table 2-5).

An issue unresolved based on the available studies is that the viral units used in the feeding studies were reported as focus-forming units (FFU) rather than individual viral particles (WERF, 2011). FFU assays determine virus titer by fluorescent staining of the viral antigen expressed in cells during infection (Keiser et al., 2021). The most commonly used assumption—that plaque-forming units (PFUs) of rotavirus are equivalent to the FFUs from the feeding study—could overestimate the risk associated with rotavirus (WERF, 2011) because the FFUs reported in the feeding study might have contained more than one PFU per FFU. PFUs can arise from more than one virus particle.

Campylobacter jejuni. Teunis et al. (2018) provided a multilevel model utilizing data from a set of different volunteer challenge studies and outbreak data on *C. jejuni*. Challenge studies have not included low doses because the primary objective of the studies was to achieve high illness rates to assess interventions. Outbreak data are from situations where contaminated raw milk was consumed. Neither situation is optimally reflective of the types of low-dose water matrix exposures that would be relevant for recreational water exposures. Teunis indicates that it appears that in the *C. jejuni* challenge studies, the doses required for infection and the doses required for causing symptoms (illness) have different orders of magnitude, so it might be concluded that a high dose is needed to cause illness. The dose-response parameters in Table 2-5 were used in conjunction with a conditional probability of illness, as discussed below.

E. coli O157:H7. The *E. coli* O157:H7 dose-response model was derived using data from eight outbreaks (Teunis et al., 2008b) and from an assumption that doses ingested in each of those outbreaks were Poisson-gamma distributed. The exposure model was refined by adjusting the gamma distribution parameter for exposure to reflect the dispersion associated with each outbreak. An exploration of various models led Teunis et al. (2008b) to select a beta-Poisson dose-response model (infection endpoint). Teunis developed and made available 10,000 pairs of dose-response parameters. The point

estimate values are reported in the paper as best estimates; however, using the 10,000 α and β pairs is recommended whenever possible.

Teunis et al. (2004) also published a dose-response model for *E. coli* O157:H7 for a single outbreak that occurred in Japan. A beta-Poisson model was also used in that study, and dose-response parameter values were developed separately for children and adults. However, a single outbreak provides limited information because the dose range is usually small, not several orders of magnitude as in experimental studies.

The recommended dose-response model in this TSM from Teunis et al. (2008b) was selected because it is considered more comprehensive as it covers a broader range of exposure conditions. The outbreaks included in the model encompass various transmission routes, including hand-to-mouth contamination, various foods, and water. Estimated exposure in these incidents covered a range of doses, from a few bacteria to approximately 10,000, and the attack rates ranged from 0.5%–80%.

Salmonella enterica. Haas et al. (1999) estimated *Salmonella* infectivity using a beta-Poisson model and pooled data from human feeding studies conducted by McCullough and Eiselle (1951a,b,c). The studies investigated the infectivity of five *Salmonella* species (*S. newport*; *S. derby*; *S. bareilly*; *S. anatum* strains I, II, and II; and *S. maleagridis* strains I, II and III). Haas et al. (1999) determined that the beta-Poisson model best fit the pooled study data when four outliers (of 50 data points) were removed; one outlier did not appear to represent monotonic behavior, and the remaining three data points lacked consistency with adjacent doses in the same experiment. The pooled model was not statistically different from models for the individual studies and had $\alpha = 0.3126$ and $\beta = 2{,}885$ (Fazil, 1996). Haas et al. (1999) validated the model using data on a waterborne salmonellosis outbreak in California from Boring et al. (1971) and Riverside County Health Department et al. (1971).

Other researchers also have reported dose-response relationships for *Salmonella*, based on the same feeding studies (Coleman and Marks, 2000; Coleman et al., 2004), using very different fundamental assumptions. That work highlights the potential importance of strain variability, as the ID_{50}s appear to vary by several orders of magnitude. Bollaerts et al. (2009) report a generalized linear mixed model for the dose-response relationship based on a series of foodborne outbreaks. These outbreaks demonstrate that the vehicle of transmission can be important (i.e., different types of food) and that a higher level of infectivity at substantially lower doses than those reported by Haas et al. (1999) is possible. Although a substantial amount of uncertainty is associated with the potential importance of strain variability in the dose-response relationship reported by Haas et al. (1999), it is widely used in QMRA. Strain variability is recommended in this TSM because it is based on human feeding study data and covers a wide range of potentially important environmental strains of *Salmonella*.

Cryptosporidium sp. The dose-response model for *Cryptosporidium* is based on the analysis conducted for the Long Term 2 Enhanced Surface Water Treatment Rule (LT2ESWTR) (U.S. EPA, 2005). The LT2ESWTR analysis includes feeding study data from six isolates of *Cryptosporidium.* Those isolates included Iowa (DuPont et al., 1995; Okhuysen et al., 1999), TAMU (Okhuysen et al., 1999), UCP (Okhuysen et al., 1999), Moredun (Okhuysen et al., 2002), TU502 (Chappell et al., 2006), and 16W. In the experimental dose-response studies, human response to different isolates of *Cryptosporidium parvum* varied widely (Okhuysen et al., 1999, 2002; Messner et al., 2001). With analyses based on Messner et al. (2001), the LT2ESWTR *Cryptosporidium* dose-response model was developed using Bayesian analyses of individual and combined data sets for different isolates and outbreak data. The LT2ESWTR dose-response model is exponential with model parameter $r = 0.09$. Uncertainty within the

dose-response model is evaluated by allowing the model parameter to vary uniformly across the range of 0.04 to 0.16, consistent with the range reported in the LT2ESWTR (U.S. EPA, 2005). Several researchers previously reported dose-response relationships for *Cryptosporidium (*Haas et al., 1996; Okhuysen et al., 1999; Teunis et al., 2002a,b; Englehardt and Swartout, 2004, 2006; WHO, 2009). The EPA's dose-response model for *Cryptosporidium* is used for this TSM because it accounts for all strains of *Cryptosporidium* reported in the literature and is the most comprehensive analysis reported. More recently, Messner and Berger et al. (2016) reported on the application of a fractional Poisson dose-response model that functions as an upper bound to the exponential model and assumes that all oocysts are capable of initiating infection (an assumption that may not describe risks from zoonotic sources of *Cryptosporidium*).

Giardia lamblia. The *Giardia lamblia* dose-response model used in this TSM has been used in other published QMRA (Regli et al., 1991; Rose et al., 1991; Rose and Gerba, 1991; Teunis et al., 1996; Haas et al., 1999). In this dose-response model, *Giardia* infectivity is estimated using data from previously conducted human feeding studies conducted by Rendtorff (1954a,b) and Rendtorff and Holt (1954a,b). In the studies, volunteers were fed doses of one to 10^6 *Giardia* cysts, and infection was defined as cyst excretion in the feces. Rose and Gerba (1991) estimated infectivity using an exponential dose-response model, assuming a Poisson distribution of *Giardia*, and calculated r = 0.0199 (Table 2-5).

2.1.1.3.3 Morbidity

Morbidity is a term used to describe the proportion of infections that progress to a symptomatic response (illness) (U.S. EPA and USDA, 2012). For each of the reference pathogens, morbidity is expressed as a range to the extent that supporting data are available. Table 2-6 summarizes the parameter values used to characterize the proportions of infections that result in illness. The EPA presents point estimates and ranges for some of the reference pathogens to define a distribution for input into the risk calculations. Narrative descriptions of the justification for the selected values follow.

Adenovirus. Teunis et al. (2016) provide point estimates to be used in the dose-dependent infection-to-illness rate for adenovirus. The values provided in Table 2-6 are integral to the dose-response model chosen in Table 2-5. Teunis et al. (2016) provided the η and *r* point estimates, so Bayesian modeling is not required for running the model.

Norovirus. Teunis et al. (2020) highlighted previously reported results that the conditional probability of illness in an infected individual may also depend on the dose to which that the individual was exposed (Teunis et al., 1999). Thus, at high doses, the risk that an infection is symptomatic could be higher than at low doses (Teunis et al., 2008a). Teunis et al. (2020) provide a concise description of the posterior dose-response parameter distributions comprising a vector of mean values and a covariance matrix defining a bivariate normal distribution for the illness dose-response parameters.

Rotavirus. The CDC (2012) reports that infection with rotavirus is nearly universal, with almost all children infected by five years of age. Recovery from a first rotavirus infection usually does not lead to permanent immunity. After a single natural infection, 38% of children are protected against any subsequent rotavirus infection, 77% are protected against rotavirus diarrhea, and 87% are protected against severe diarrhea. Reinfection can occur at any age. Subsequent infections confer progressively greater protection and are often less severe than the first. Recurrent rotavirus infections affect people of all ages. Recurrent infections are usually asymptomatic or result in mild diarrhea that can be

Table 2-6. Literature-reported values for the proportion of infections resulting in illness from exposure to reference pathogens.

Reference pathogen	Parameter point estimate	Parameter description	Reference
Rotavirus	0.4[a]	Midpoint of the range [0.1 to 0.7]	Haffejee et al., 1995 Ward et al., 1986a,b
Norovirus	η = 0.801 r = 3.19	Point estimate values for dose-dependent relationship[b]	Teunis et al., 2020
	w = (mean = 1.74, variance = 5.55, covariance = −0.708) z = (mean = 1.82, variance = 4.64, covariance = −0.708)	Intermediate parameters to derive a distribution of η and r	
Adenovirus	η = 6.53 r = 0.41	Point estimate values for dose-dependent relationship[b]	Teunis et al., 2016
Cryptosporidium spp.	0.5[a]	Midpoint of the range [0.2 to, 0.7]	U.S. EPA, 2006a
Giardia lamblia	0.45[a]	Midpoint of the range [0.2 to 0.7]	Eisenberg et al., 1996
Campylobacter jejuni	η = 0.88 r = 0.06	Point estimate values for dose-dependent relationship[b]	Teunis et al., 2018
	w = (mean = −2.744, variance = 1.337, covariance = 0.010) z = (mean = -4.89×10^{-3}, variance = 0.993 covariance = 0.010)	Intermediate parameters to derive a distribution of η and r	
E. coli O157:H7	0.4[a]	Midpoint of the range [0.2 to 0.6]	Bielaszewska et al., 1997 Ludwig et al., 2002 Werber et al., 2008
Salmonella enterica	0.285[a]	Midpoint of the range [0.17 to 0.4]	Teunis et al., 1999

Notes:

a. Parameter values are probabilities, where 1 = 100%, so 0.6 means 60% of infections result in illness. For pathogens where a point estimate is given, the EPA assumed that illness is independent of dose.

b. Illness is assumed to be dependent on dose; η and r are point estimates used to model the dose-dependent illness.

preceded or accompanied by vomiting and low-grade fever. Based on these data, approximately 62% are susceptible to subsequent rotavirus infection, and the overall probability of symptomatic response to a nonneonatal rotavirus infection is no greater than approximately 23% (CDC, 2012).

In the feeding study reported by Ward et al. (1986a,b) on which the dose-response relationship is based, 17 of 30 (57%) infected subjects became ill with doses equivalent to that required for infection. Haffajee et al. (1995) noted that diarrhea has been reported to occur in 11%–70% of adults infected with rotavirus. Based on these reported data, for the purposes of this TSM, the range of infections leading to illness is estimated to be 0.1–0.7.

Campylobacter jejuni. While infection induced by a pathogen may be asymptomatic, illness symptoms can be considered conditional on infection; in the absence of infection, illness is assumed not to have occurred. For *Campylobacter jejuni*, Teunis et al. (2005, 2018) contend that there appears to be evidence indicating a dose-dependent illness response among infected subjects. Teunis et al. (2018) calculated the (unconditional) probability of illness as the product of the probability of infection and the conditional probability of illness given infection. The paper uses empirical data from previous studies to derive a mathematical relationship that describes the conditional probability of illness given infection as a function of ingested dose.

E. coli O157:H7. The progression from infection to symptomatic illness for *E. coli* O157:H7 is assumed to be in the range of 0.2–0.6 based on outbreak data (Bielaszewska et al., 1997), the percentage of symptomatic and asymptomatic individuals who were household contacts of hemolytic uremic syndrome patients (Werber et al., 2008), and the occurrence of anti-Stx2 IgG (Ludwig et al., 2002). This range is consistent with the proportion of illnesses reported in an analysis of an *E. coli* O157:H7 outbreak (Teunis et al., 2004).

Salmonella enterica. The progression from infection to symptomatic illness for *Salmonella enterica* is assumed to be in the range of 0.17–0.4. Teunis et al. (1999) report the response of human volunteers from a human feeding study using *Salmonella enterica* serovar Meleagridis (McCullough and Eisele, 1951a). The *Salmonella* isolate was produced from market samples of high moisture, spray-dried, whole egg powder, and administered in a glass of eggnog to healthy male prisoners (4 doses tested). Three strains were originally tested, and Teunis et al. (1996) reported the results for strain II, the most virulent. Fecal samples were taken daily for culturing to determine infections, and illness was defined as having diarrhea. At the two lowest doses, 1.58×10^5 organisms and 1.5×10^6 organisms ingested, one of six and five of six individuals were infected, respectively, but none became ill. At the third dose, 7.675×10^6 organisms ingested, all participants (six) were infected, and one became ill (17% of those infected). At the highest dose, 1.0×10^7 organisms ingested, five of six individuals were infected, and two became ill (40% of those infected).

Cryptosporidium sp. The progression from infection to symptomatic illness for *Cryptosporidium* is based on research to develop the LT2ESWTR (U.S. EPA, 2005, 2006a). In that analysis, the available literature and identified studies were analyzed. DuPont et al. (1995) found that 39% of those infected had clinical cryptosporidiosis. Haas et al. (1996) provided information based on the same data also suggesting a morbidity rate of 39% but computed 95% confidence limits of 19% and 62%. More recently, a study found that after repeated exposure to *C. parvum* (IOWA strain), the morbidity rate was the same as for the initial exposure in reinfected subjects (Okhuysen et al., 1998). Okhuysen et al. (1998) also found that 58% of their subjects who received *Cryptosporidium* doses developed diarrhea, an underestimate of morbidity because symptoms other than diarrhea contribute to the morbidity rate. Based on these data, the progression from infection to symptomatic illness for *Cryptosporidium* is assumed to be in the range of 0.2–0.7.

Giardia lamblia. *Giardia* infection is often asymptomatic, with asymptomatic cases representing as much as 50%–75% of infected persons (Mintz et al., 1993). In a study at the Swiss Tropical Institute, 27% of 158 patients who had *Giardia* cysts in their feces exhibited symptoms (Degremont et al., 1981). Based on these data, the progression from infection to symptomatic illness for *Giardia* is assumed to be in the range of 0.2–0.7. This range is consistent with that reported by Eisenberg et al. (1996), who

summarized the proportion of infections by *Giardia lamblia* that result in illness as reported in Flanagan (1992), Birkhead and Vogt (1989), and Dupont and Sullivan (1986).

2.1.2 Surrogates: Fecal Indicator Bacteria

For over a century, culture-enumerated FIB have facilitated important gains in public health protection against pathogens in drinking and recreational waters that are associated with disease. Due to the potential diversity of pathogens associated with fecal contamination and their variable occurrence, often at low densities, in environmental waters, routine pathogen monitoring can be costly, technically challenging, and not feasible in some circumstances (Korajkic et al., 2018). Instead, public health agencies monitor levels of surrogates—the FIB—in waters to measure the extent of fecal contamination. Elevated levels of FIB have a low correlation with the presence (or absence) of specific pathogens (Savichtcheva and Okabe, 2006; Wu et al., 2011). However, FIB are highly abundant in the intestinal tract and feces of warm-blooded animals and humans. FIB co-occur with enteric pathogens in the feces of infected animals and humans, so their use as metrics of fecal contamination in ambient waters can indirectly protect the public from exposure to the waterborne pathogens associated with fecal contamination. Their use as indicators of disinfection efficacy during wastewater treatment has supported the reduction of bacterial pathogens, which can cause diseases like cholera and typhoid fever, in effluent discharges. Additionally, epidemiological studies have established a statistically significant association between increasing FIB abundance and gastrointestinal illness in some types of waters (Prüss, 1998; Wade et al., 2003; Zmirou et al., 2003; Ahmed et al., 2018).

Currently, culture-enumerated *E. coli* and enterococci are commonly recommended by public health agencies worldwide as the primary indicators of fecal contamination. Previous fecal indicator groups, such as total coliforms and fecal coliforms, have been used and are still being used in some areas. The EPA's 2012 RWQC recommended water quality values for culture-enumerated *E. coli* and enterococci, associated with two specific target illness rates, to protect primary contact recreation in fresh and marine waters (U.S. EPA, 2012).

The underlying epidemiological data supporting the EPA's RWQC were collected in waters receiving human fecal contamination, such as poorly or partially treated sewage and treated effluent discharges (Cabelli, 1983; Dufour, 1984; Wade et al., 2006, 2008, 2010). Elevated FIB levels in ambient waters lacking direct point source impacts have not demonstrated a significant potential for adverse human health effects linked to the abundance of FIB (Calderon et al., 1991; Colford et al., 2007, 2012). Eel grass beds, drying kelp (wrack), green algae (*Cladophora*), other aquatic vegetation, biofilms, sands, and sediments are examples of potential nonfecal sources of FIB (U.S. EPA, 2010b; Stewart et al., 2008; Fujioka and Byappanahalli, 2003; Yan et al., 2011; Byappanahalli et al., 2012). Nonfecal sources of FIB can potentially contribute substantial FIB loads to a waterbody (Byappanahalli et al., 2006, 2012; Ksoll et al., 2007; Litton et al., 2010; Skinner et al., 2010). Data demonstrate that these bacterial sources are not usually sources of fecal pathogens (Byappanahalli et al., 2006, 2012).

Although recreational water epidemiological studies conducted in waters where the predominant impacts are from nonhuman or nonpoint sources have not provided clear, consistent associations between FIB and adverse human health outcomes, a growing body of QMRA-based studies suggests animal fecal sources can cause fewer diseases and pose less risk than human fecal contamination (Schoen and Ashbolt, 2010; Soller et al., 2010b, 2015; WERF, 2011; McBride et al., 2013). Human health risks from recreational exposures can be quite low when point sources, such as secondary treated and disinfected WWTP effluent, do not impact a waterbody (Soller et al., 2016). Pathogens of

public health concern from different fecal sources also can differ relative to the level of FIB measured in environmental waters (U.S. EPA, 2009a,b, 2010b; Soller et al., 2015). The following sections provide information on the abundance of *E. coli* and enterococci in animal feces and discuss the fate and transport characteristics of these FIB in environmental waters.

2.1.2.1 Abundance of *E. coli* and Enterococci in Animal Feces

The FIB, enterococci and *E. coli,* are abundant in the feces of warm-blooded animals, although the reported range for each FIB varies among different fecal sources (Table 2-7). Generally, the range of FIB is from 10^5 to 10^{10} per gram (g) of feces in humans, pigs, chickens, and gulls. When considered alongside the range of reference pathogens in the context of the health modeling, the level of FIB at a specific target illness level can be different for different fecal sources. Similar to the pathogen ranges, the ranges shown in Table 2-7 can be included in the QMRA modeling as a distribution between the high and low values, with each value being equally likely (i.e., uniformly distributed).

Table 2-7. Literature-reported density of FIB in human and selected animal fecal waste.

Fecal source			Density[a] of:			
			Enterococci		*E. coli*	
			min[b]	max[c]	min	max
Human	Raw sewage	Range[d]	5.8	8.0	6.7	8.0
Human	Secondary treated and chlorinated effluent	Range[d]	0.5	2.7	0.5	4.0
Animal	Cattle	Range[d]	2.0	5.1	5.0	6.7
Animal	Cattle	Basis[e]	D		W	
Animal	Cattle	Type[f]	C		C	
Animal	Chickens	Range[d]	5.0	7.0	5.1[g]	10.9
Animal	Chickens	Basis[e]	W		W	
Animal	Chickens	Type[f]	C		C	
Animal	Chickens	Manure[h]	L		L	
Animal	Gulls	Range[d]	4.114	9.447	3.934	10.301
Animal	Pigs[i]	Range[d]	5.3	7.2	6.1	7.3
Animal	Pigs[i]	Type[f]	D		C	

Notes:

a. Represent power of 10 exponential value (e.g., n = 10^n).

b. Denotes minimum observed value.

c. Denotes maximum observed value.

d For raw sewage and secondary chlorinated effluent, units of minimum and maximum observations are CFU per L; for livestock wastes, units are CFU per g.

e. Basis refers to the weight basis for manure. D denotes dry weight, and W denotes wet weight.

f. Sample type is either composite (C) or direct (D).

g. Reported as mean per state. Range presented is the range from the state with lowest mean density to the state with highest mean density.

h. Chicken manure type is litter (L) or fresh (F).

i. All pig fecal abundances reported are for solid, fresh fecal samples (not slurries or treated manure).

It is important to note that there are potential nonfecal sources of FIB, including autochthonous *E. coli* and enterococci found in sand, soil and sediments, aquatic and terrestrial vegetation, and ambient waters (Byappanahalli et al., 2006, 2012; Ferguson and Signoretto, 2011). Multiple studies have demonstrated that FIB are widely distributed in nature, including in habitats with little or no input of human or animal fecal contamination (Byappanahalli et al., 2012). Research has also demonstrated these FIB can persist for extended periods in sands and other reservoirs (Byappanahalli et al., 2006). Under certain circumstances, such as wet weather, the mobilization of FIB in nonhuman fecal sources and environmental (nonfecal) FIB can occur. Nonfecal indicator loading can be a significant proportion of the indicator enumerated. Enumeration methods routinely used to measure the FIB do not distinguish between FIB from fecal and nonfecal sources or human and nonhuman fecal sources.

The values shown in Table 2-7 for raw sewage, secondary treated and chlorinated effluent, cattle, chickens, and pigs are from Soller et al. (2010b). The values for gulls are from Goodwin et al. (2017).

2.1.2.2 Environmental Fate and Transport

Upon entering ambient waters, multiple environmental abiotic and biotic factors can affect the fate and transport characteristics of FIB (Korajkic et al., 2018). The primary stressors affecting the persistence of FIB in ambient waters include sunlight, rainfall, temperature, salinity, starvation (available nutrient levels), predation and competition, and disinfection (if discharged via a point source) (U.S. EPA, 2010b; Byappanahalli et al., 2012; Korajkic et al., 2018). These environmental parameters may have different and potentially significant effects on the presence and persistence of FIB depending upon the climate (tropical, subtropical, or temperate) (U.S. EPA, 2010b). FIB can partition within the water column and to sediment. Once in the sediment, the FIB can be resuspended and move further “downstream.” Beach sand is recognized as a potential reservoir of FIB and pathogens (Whitman et al., 2014). The source of the FIB can also affect the ability of FIB to persist in the environment, with increased persistence noted in FIB that originates from ruminants (Korajkic et al., 2018).

Sunlight, particularly the UV component, can increase the decay rates of FIB. FIB not associated with particles, such as organic matter, decay faster than FIB associated with particles (Gutiérrez-Cacciabue et al., 2016; Boehm et al., 2019). Gutiérrez-Cacciabue et al. (2016) also noted that the measurable DNA of FIB persisted longer than the culturable cells. Culturable FIB are known to enter a VBNC state in ambient environments, allowing increased persistence and potential regrowth when conducive conditions allow (Ferguson and Signoretto, 2011; Li et al., 2014).

Eregno et al. (2018) characterized the decay rates of FIB in seawater samples from different depths. Decay rates for FIB were low at low water temperatures (e.g., 4 degrees Celsius [°C]). At 20 °C, decay rates for FIB were higher than adenovirus of coliphages. The authors state that accounting for different decay rates at different temperatures, water qualities, and meteorological conditions in hydrodynamic modeling can improve input parameters for QMRA (Eregno et al., 2018).

The EPA conducted a literature review to identify studies that characterized various fate and transport factors affecting the persistence of FIB in ambient waters (U.S. EPA, 2010b). The review did identify multiple studies that provide more discussion on these factors.

2.2 Problem Formulation

This section describes and discusses the major factors considered for evaluating risk from exposure to recreational waters contaminated by predominantly nonhuman fecal sources. Typically, the problem formulation stage is considered the analytical phase of a risk assessment in which "the purpose for the assessment is articulated, the problem is defined, and a plan for analyzing and characterizing risk is determined" (U.S. EPA 1998b, 2014d). In this TSM, the factors considered (e.g., target illness level, exposed population) and the process used for evaluating human health risks from pathogen exposure in recreational waters are consistent with the EPA's 2012 RWQC. The EPA has included the selection of risk assessment parameters (e.g., reference pathogens, exposure profiles) in the health modeling presented here to facilitate a consistent approach for evaluating risk from nonhuman fecal sources. Other risk management and technical considerations may be included if they are clearly identified and transparently described in alternative WQC developed using this guidance.

2.2.1 Conceptual Model

A conceptual model was developed to provide useful information that characterizes and communicates the potential health risks related to exposure to fecal contamination in recreational waters. The model depicts the sources of the pathogens in these waters, the recreational routes of exposure for potentially sensitive biological receptors of concern, and the potential adverse health effect endpoints (e.g., gastrointestinal illness, respiratory illness) (Figure 2-5). Note that the conceptual model is the same for different sources of fecal contamination. Recreational waters can receive fecal loading from human and nonhuman sources.

2.2.1.1 Description of Conceptual Model (Figure)

The conceptual model is intended to explore potential relationships between exposure to a contaminant or stressor and the adverse health endpoints important for management goals. In the case of this TSM, the recommended 2012 RWQC can be adjusted to account for waters affected by predominantly nonhuman fecal sources. Because of the linkage to the RWQC, aspects of the conceptual model and the analysis discussed below are constrained by the decisions and assumptions underlying the RWQC, such as:

1. Primary contact recreational scenario: The RWQC was designed to protect the primary contact recreation designated use. Incidental ingestion while recreating is considered the predominant exposure pathway for primary contact recreation.

2. General Population: The values recommended in the RWQC are informed by epidemiological data collected from the general population (i.e., study participants of all ages combined). The QMRA modeling discussed in Sections 2 and 3 includes exposure data for the general population. Section 4 presents a sensitivity analysis comparing exposure for the general population compared to children.

3. Gastrointestinal illness: The target illness rates discussed in the RWQC are based on the gastrointestinal illness endpoint.

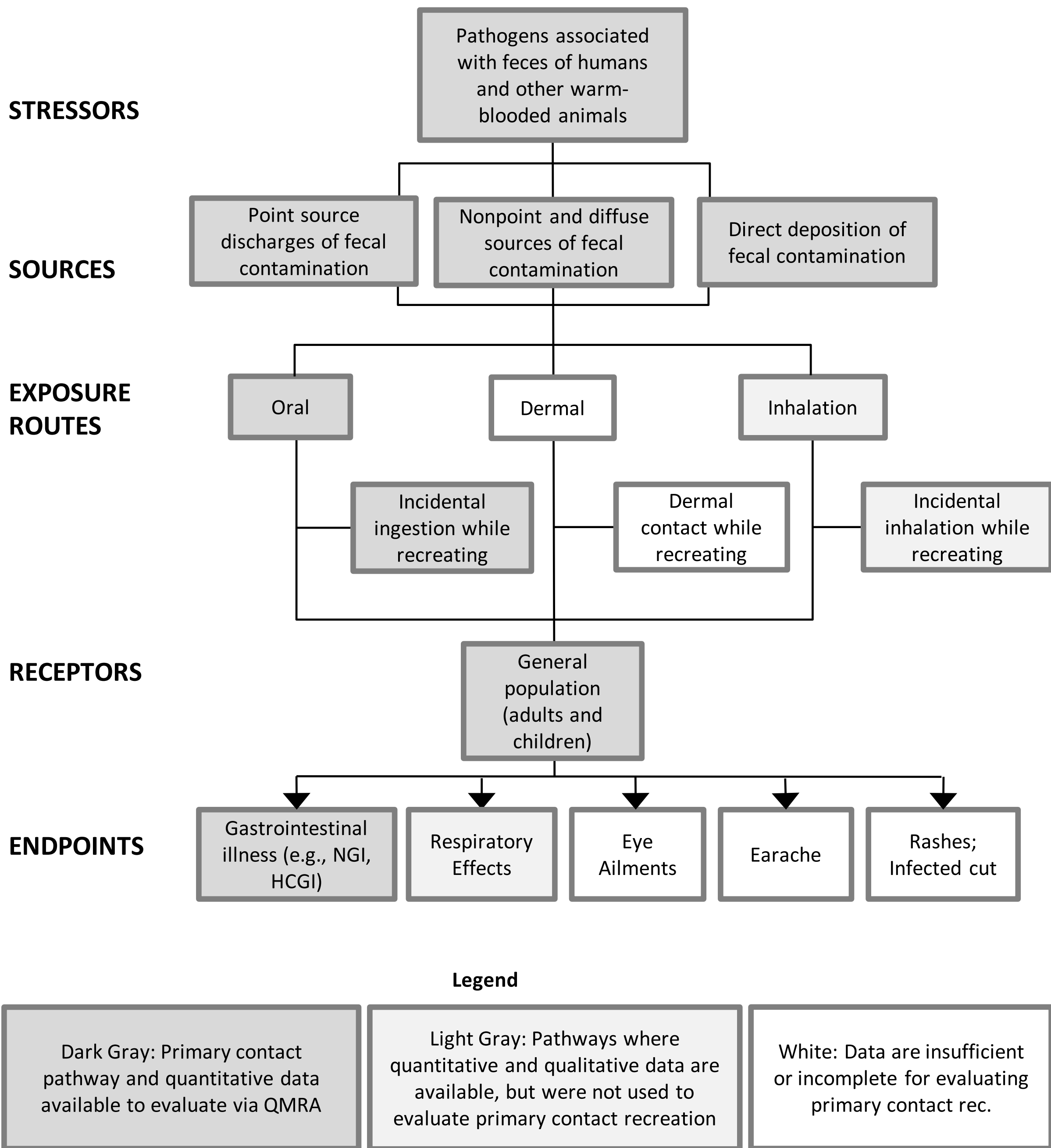

Figure 2-5. Conceptual model of exposure pathways to enteric pathogens associated with fecal contamination in surface waters while recreating.

4. Boxes associated with the different components of the conceptual model shown in Figure 2-5 are shaded to signify the primary scenario discussed in this TSM to adjust the RWQC for predominantly nonhuman fecal sources. Dark gray boxes denote the primary contact pathway and include quantitative parameter data presented below to evaluate via QMRA. Lighter gray boxes denote the inhalation exposure pathway where quantitative and qualitative data are available but were not used to evaluate primary contact recreation. Inhalation exposure is less than incidental ingestion (Backer et al., 2010; Butler et al., 2012). White denotes the dermal exposure pathway and includes items for which data are insufficient or incomplete for evaluating primary contact recreation.

2.2.1.2 Conceptual Model to Define Scope of Assessment

2.2.1.2.1 Factors Included in the Conceptual Model

Stressors

The stressors are pathogens associated with the feces of humans and animals that enter surface water from various contamination pathways. Feces-associated, also referred to as enteric, pathogens of public health concern belong to three major groups: viruses, bacteria, and protozoa. Eight reference pathogens, including representatives from each pathogen group accounting for more than 97% of nonfoodborne illnesses in the United States, are considered as part of the QMRA framework in this TSM. Section 2.1.1 describes the reference pathogens and why each was selected.

Sources

Sources of fecal contamination can be characterized as point and nonpoint sources that contaminate surface waters via direct and/or indirect pathways. Point sources of feces include WWTPs that treat human sewage and subsequently discharge treated effluent directly to a river, lake, or coastal waterbody. Large farms that raise livestock, such as cows, pigs, and chickens, known as CAFOs,[10] also can be considered point sources of fecal contamination (NOAA, 2022). Untreated animal waste from CAFOs can enter nearby waterbodies as raw sewage. Nonpoint sources of feces include more diffuse sources of fecal contamination, such as septage from septic systems, stormwater runoff from urban, suburban, or agricultural areas, inputs from wildlife, including avian, and leaky infrastructure. Generally, nonpoint fecal sources can be associated with wet weather mobilization leading to overland runoff or subsurface flow of fecal pathogens to a waterbody. Some sources, such as shorebirds and livestock with access to surface waters, can contribute feces via direct deposition. The potential loading of fecal material from direct deposition from livestock operations can sometimes have a relevant public health concern similar to the risks posed by human contamination (Soller et al., 2010b).

Routes of Exposure

Exposure to enteric pathogens in recreational water can occur via oral exposure (i.e., incidental ingestion while recreating); dermal exposure (i.e., contact of exposed parts of the body with water during recreational activities such as swimming, wading, or water skiing); and inhalation exposure to aerosols (i.e., while recreating). Epidemiological data used to support the 2012 RWQC found that (1) swimmers reported more illness than nonswimmers; (2) there were increased adjusted odds ratios

[10] The EPA defines CAFO as any animal feeding operation with more than 1,000 animal units confined on-site for more than 45 days during the year. Any animal-feeding operation discharging manure or wastewater into a waterbody is considered a CAFO, regardless of size (https://www.epa.gov/npdes/animal-feeding-operations-afos).

with increasing levels of body exposure to water at the beach for recreators reporting body and head immersion, a surrogate for incidental ingestion; and (3) swimmers who swallowed water reported the highest rate of illness incidence. For consistency with the RWQC, incidental ingestion while recreating is considered the primary route of exposure in this TSM. Incidental ingestion parameter values are discussed below for use in QMRA.

Receptors

Humans who recreate in a water body where fecal contamination is present could be exposed to enteric pathogens through ingestion, dermal contact, and inhalation of aerosols while recreating on or in contaminated surface waters. The EPA relied on recreational water epidemiological studies that characterized health effects for the general population to inform the 2012 RWQC. To be consistent with the RWQC, this TSM includes QMRA parameters for the general population of recreators.

Endpoints

Published epidemiological and QMRA results and outbreak reports provide information about associations between exposure to recreational waters containing fecal contamination and illness in recreators. Common illness endpoints and their definitions include (Cabelli, 1983; Dufour, 1984; Kay et al., 1994; Prüss, 1998; Wade et al., 2003, 2006, 2008, 2010; U.S. EPA, 2012; Fewtrell and Kay, 2015):

1. Gastrointestinal:[11] any of the following within 10–12 days after swimming: (a) diarrhea (three or more loose stools in a 24-hour period), (b) vomiting, (c) nausea and stomachache, or (d) nausea or stomachache and impact on daily activity;
2. Respiratory: any two of the following: sore throat, cough, runny nose, cold, or fever;
3. Rashes: a rash or itchy skin;
4. Eye ailments: either an eye infection or a watery eye;
5. Earaches: ear pain, ear infection, or runny ears; and
6. Infected cut: a cut or wound that became infected.

Gastrointestinal illness is a commonly observed endpoint in health studies conducted at beaches affected by fecal contamination. In epidemiology studies, significant increases for other illness endpoints among swimmers are less commonly reported. The epidemiological data used to inform the 2012 RWQC identified gastrointestinal illness as the most sensitive endpoint among swimmers participating in the studies. The fecal-oral route in the transmission of waterborne disease is an important factor associated with exposure to recreational waters affected by fecal contamination (de Graaf et al., 2017). Pathogens associated with feces can initiate colonization and infection in the gastrointestinal tract of a new host (Clements et al., 2012; Kolling et al., 2012; Stecher, 2015). For consistency with the RWQC, this TSM focuses on gastrointestinal illness as the primary adverse health outcome among recreators.

[11] Case definitions of gastrointestinal illness differ and have changed over time. Two common definitions in use include HCGI and NGI. Fever is included as a required symptom for HCGI, but not for NGI.

2.2.1.2.2 Factors not included

Recreating children can be at increased risk from exposure to fecal contamination because they spend more time in contact with the water compared to adults, they incidentally ingest more water than adults while recreating, and they can be more susceptible to infection and illness due to their immature immune systems (Dufour er al., 2017; Wade et al., 2022). To be consistent with the 2012 RWQC recommendations tied to illness rates reported in the general population, which includes children, this TSM describes the general population of receptors within the QMRA framework presented. However, the EPA evaluates and discusses differences between lifestages in Section 4.1.2.1 of the Effects Characterization. Data for incidental ingestion by children are included in the sensitivity analysis (Section 4.2.2).

2.2.1.2.2.1 Rationale for Not Considering Other Endpoints

Although the RWQC and this TSM focus on gastrointestinal illness as the primary health endpoint related to incidental ingestion exposure to fecal contamination in recreational waters, other endpoints may result after exposure and have been reported in some health studies. For example, in an epidemiological study conducted by Sinigalliano et al. (2010) at a nonpoint source affected beach, an increase in gastrointestinal, respiratory, and skin illnesses was reported by recreators, and a significant association between exposure and skin illnesses and culturable enterococci was observed. Some pathogens can initiate infection at more than one site in the body depending on exposure, susceptibility, and the type of pathogen. Human adenovirus infection is known to cause respiratory and eye infections in addition to gastrointestinal illness (Radke and Cook, 2018). Ear infection can occur in the outer ear, otitis externa, or the inner ear (due to a wider sinus or upper respiratory infection).

These other illnesses generally occur at a lower rate than gastrointestinal illness (Fleisher et al., 1998; McBride et al., 1998; Haile et al., 1999; Wade et al., 2008). For example, Wade et al. (2008) reported a mean overall gastrointestinal illness incidence of 7.3%, upper respiratory infection incidence of 5.7%, rash incidence of 2.7%, and eye irritations and infections of 2.9%. Kay et al. (1994) and Fleisher et al. (1998) reported 14.8% gastrointestinal illness in swimmers and 9.7% in nonswimmers, 4.7% incidence of respiratory infection in swimmers and 3% in nonswimmers, and 4.2% incidence of ear ailments in swimmers and 4.8% in nonswimmers. Additionally, significant associations between these other endpoints and FIB are less commonly reported in epidemiological studies.

While users of this TSM can consider other health endpoints in a QMRA, it is recommended that the gastrointestinal endpoint be considered to facilitate consistent comparisons with the recommended RWQC.

2.2.1.2.2.2 Other potential exposure routes

Exposure to enteric pathogens in contaminated recreational waters can occur via inhalation and dermal contact. Inhalation can occur from exposures to wave-associated aerosols, personal watercraft, and boat spray. Respiratory illness has been reported in epidemiology studies conducted at beaches affected by human fecal contamination, although at lower rates than gastrointestinal illness (Kay et al., 1994; Wade et al., 2008; Sinigalliano et al., 2010). Dermal exposure can occur through recreational water contact. Although one of the skin's main functions is to provide a physical barrier to external hazards, recreators with breaks in the skin can be at risk of infections at those sites (Oliver, 2005). A caveat to this point is that many bacteria can be considered opportunistic pathogens, and wound infection is possible from microorganisms that are not typically considered pathogenic. Additionally,

some naturally occurring pathogens in aquatic environments, such as *Vibrio vulnificus*, are not associated with fecal contamination. *Vibrio* wound infections can be serious (CDC, 2022).

Except for the published adenovirus dose-response function, which is characterized for respiratory infections, the other dose responses included in this TSM are specific for gastrointestinal infections. For QMRA discussed in this TSM, the dose response for adenovirus is assumed to be the same for both respiratory and gastrointestinal infections.

2.2.2 Analysis Plan

The QMRA method for the human health risk evaluation discussed in this TSM is a static, stochastic Monte Carlo simulation-based approach selected because it is relatively simple, straightforward, and parsimonious compared to dynamic modeling (Soller et al., 2008). In this type of analysis, individual recreational events are considered independent and treated probabilistically. Statistical distributions rather than point estimates are used to characterize input parameters. In contrast to a deterministic (point estimate-based) model, a stochastic QMRA model considers the variability associated with the input parameters and yields a distribution of outcomes rather than a single-value outcome. The Monte Carlo-based approach involves running the model multiple times; each run is called an iteration, using randomly selected values within the range of each parameter distribution included in the model (U.S. EPA, 1994, 2007d). A stochastic model will produce different results each time it is run because of statistical chance. Over the thousands of iterations conducted, a distribution of probable outcomes is generated. For example, one output of the QMRA framework in this TSM is that an estimate of the probability of illness is generated that is associated with recreational exposure to the reference pathogens. When the QMRA methodology is applied to derive the FIB level corresponding to a single target illness rate, it represents a single iteration of the model in which all inputs must be specified as static point estimates; the output represents a single FIB level corresponding to the target illness rate. The recommended QMRA approaches in this TSM are consistent with the EPA's Microbiological Risk Assessment Tools, Methods, and Approaches for Water Media (U.S. EPA, 2014b).

The EPA conducted extensive literature searches between 2007 to 2021 (Appendix A). The EPA reviewed the published literature and compiled information on the reference pathogens, exposure parameters, and health effects associated with primary contact in feces-contaminated recreational waters (U.S. EPA 2009a,b, 2010a,b, 2013a). The parameter distributions and values specified in this TSM are based on data from scientific literature. Parameters that are provided as standard values are justified below.

2.2.2.1 Measures of Effect

This TSM is focused on gastrointestinal illness as the measure of effect because it is the most common endpoint reported from recreational water epidemiological studies. The EPA's 2012 RWQC (U.S. EPA, 2012) provides the health effects basis for developing criteria expressed as culture-enumerated *E. coli* and enterococci. The recreational water epidemiological studies informing the development of those criteria were conducted in waters affected by human fecal contamination. Results demonstrated a predictive positive association between gastrointestinal illness and culturable *E. coli* and enterococci (Cabelli, 1983; Dufour, 1984). In these studies, gastrointestinal illness is expressed as NGI, which is defined as: "any of the following [within ten to 12 days after swimming]: (a) diarrhea (three or more loose stools in a 24-hour period), (b) vomiting, (c) nausea and stomachache, or (d) nausea or stomachache and impact on daily activity" (Wade et al., 2006). To facilitate comparison with the

RWQC, it is recommended that one of the two recommended target NGI illness rates (i.e., 32 or 36 NGI per 1,000 recreators) be used in the QMRA framework discussed in this TSM.

2.2.2.2 Measures of Exposure

This TSM is focused on incidental ingestion because it is the primary source of exposure during primary contact recreational activities. In HHRA, exposure is defined as human contact with a biological, physical, or chemical agent—usually through ingestion, inhalation, or dermal contact. Risk assessment can be performed for specific target populations or for an individual target organism, such as a human with a defined exposure pattern. Exposure assessment involves determining or estimating (qualitatively or quantitatively) the magnitude, frequency, duration, and route(s) of exposure (U.S. EPA, 2011b). A primary purpose of exposure estimation is to support dose estimation (U.S. EPA, 1998b). Dose is the amount of a pathogen that enters or interacts with a host (ILSI, 2000). For nearly all QMRA contexts, dose refers to potential dose (i.e., the number of pathogens ingested in a specified period) because the actual number of pathogens that an individual is exposed to is usually unknown. The dose is typically calculated as a function of the level of pathogens enumerated from a specific volume of the exposure medium (e.g., the most probable number per liter of water), and the volume of that medium that is ingested or inhaled. Ingestion of contaminated water is the main exposure pathway to enteric pathogens, although inhalation and dermal contact can also be important in certain circumstances (Hauchman, 2008). Empirical health data collected from recreational water epidemiological studies report gastrointestinal illness symptoms more frequently than respiratory or dermal illness symptoms (Wade et al., 2008, 2010).

Because this TSM serves to support the EPA's 2012 RWQC, its scope is purposefully focused on recreational exposures in ambient waters. The EPA selected incidental ingestion during primary contact activities (such as swimming) for input to the QMRA framework because (1) data suggest that incidental ingestion can be considered the highest potential exposure pathway for enteric pathogens while recreating, (2) the health relationship underpinning the RWQC is based on this scenario, and (3) the approach is consistent with the derivation of RWQC (U.S. EPA, 2019a). Dorevitch et al. (2011) studied the volume of water ingested during a range of recreational activities in the Chicago Area Waterway System (CAWS) and at a public outdoor swimming pool. Study participants took part in one of the following activities on the CAWS: canoeing, fishing, kayaking, motor boating, or rowing. In the swimming pool, participants took part in canoeing, fishing, kayaking, swimming, or wading/splashing. The study results indicate that the odds of ingesting a teaspoon or more of water are significantly higher among swimmers than among those who just immersed their head in a swimming pool or those who participated in the other, more limited contact activities on surface waters. Therefore, the EPA determined that using a swimmer scenario for exposure as the basis for the criteria is protective of these other aquatic activities.

In this TSM, oral exposure is defined by incidental ingestion by the general population participating in primary contact recreation where immersion and incidental ingestion are likely. In QMRA, incidental ingestion is an event-based parameter (i.e., the volume of water ingested per recreational event). Multiple recreational events over time are considered independent exposures. This exposure parameter approach differs from a chemical risk assessment, which is based on the ingestion rate in units of volume per day. For pathogen exposures, the exposure input value determines the dose of pathogens ingested, which is then combined with the pathogen dose-response parameters to calculate a probability of infection per event for each reference pathogen.

The EPA identified and reviewed seven studies found in the scientific literature, reporting data on incidental ingestion by recreators (U.S EPA, 2019a). Of the seven studies identified, two studies, Dufour et al. (2017) and DeFlorio-Barker et al. (2017), reported quantitative data that may be used as input parameters for QMRA. See U.S. EPA (2019a) for a more complete discussion of the strengths and limitations of the seven studies identified in the literature.

Dufour et al. (2017) used cyanuric acid as an indicator of the amount of water ingested while swimming in an outdoor pool. Cyanuric acid acts to stabilize hypochlorite levels in pool water exposed to sunlight. Ingested cyanuric acid is not metabolized and excreted in urine. Pool water samples were collected before the start of swimming activities, and urine from 549 participants, aged six to 81 years and approximately evenly divided by gender and lifestage, was collected for 24 hours after the swimming event ended; pool water and urine samples were analyzed for cyanuric acid. Study results reported that males ingested more than females and younger children ingested more than older children or adults. For the general population, the average amount of water ingested was 32 mL (range: 0 mL–280 mL) (Dufour et al., 2017).

While Dufour et al. (2017) report quantitative data for water ingested while recreating, estimates were gathered from participants recreating in a swimming pool. There is uncertainty associated with how recreating in a swimming pool might compare with recreational behavior in ambient waters. Separate ingestion estimates were not provided for participants with varying degrees of body exposure (e.g., waist-deep, submerged head). The distribution of ingestion reported by Dufour et al. (2017) did not differ significantly from the distribution reported previously in a pilot-scale study by Dufour et al. (2006).

DeFlorio-Barker et al. (2017) combined ingestion data from Dufour et al. (2017) and participant-estimated time spent in the water data from 12 cohorts of previously conducted epidemiological studies at fresh and marine beaches to provide an estimate of the volume of water ingested per swimming event. The NEEAR Study and Southern California Coastal Water Research Project (SCCWRP) epidemiological studies included 68,685 recreators at four freshwater and eight marine beaches. The study participants estimated how much time they spent in the water. DeFlorio-Barker et al. (2017) combined the volume of ingestion from Dufour et al. (2017) and the self-reported time spent in the water by the epidemiological study participants to simulate an estimated distribution of the volume of water ingested per event. The DeFlorio-Barker study reported similar trends to Dufour and other ingestion studies, including that males ingested more than females and younger children ingested more than older age groups. The similar trends (e.g., gender and age-related incidental ingestion) reported by both studies may indicate that recreational behavior between swimming pools and ambient waters may not be dramatically different. While the larger dataset provides more participants by gender and age, the underlying ingestion data comes from the swimming pool study conducted by Dufour et al. (2017). The estimated distribution assumes a linear relationship between the volume of water ingested and the total time spent in the water (DeFlorio-Barker et al., 2017). However, it is possible that some individuals may have more exposure in less time if the behavior includes head immersion activities compared to other individuals who wade for a longer time in knee-depth or waist-deep water. The epidemiological studies included self-reported data of swimming exposures and time spent in the water that may be subject to recall bias (DeFlorio-Barker et al., 2017).

A comparison of the mean and upper 90th percentile ingestion estimates for both Dufour et al. (2017) and DeFlorio-Barker et al. (2017) is presented in Table 2-8. For this TSM, the ingestion distribution for the general population is used as the input for exposure in QMRA because the 2012 RWQC focused on the general population. The EPA chose the general population data from Dufour et al. (2017) for the base analysis because it represents empirically measured ingestion data, whereas the ingestion results from DeFlorio-Barker et al. (2017) are modeled from estimated time spent in the water for NEEAR study participants combined with the Dufour ingestion data. See Section 4.1.2.1 in the Effects Characterization for discussion on ingestion rates by lifestage.

2.2.2.3 Expression of Criteria (GM, STV and BAV)

The EPA's 2012 RWQC recommendations consist of a magnitude, duration, and frequency of exposure. The magnitude of exposure is the numeric expression of the maximum amount of the contaminant that may be present in a water body that supports the designated use. Duration is the period over which the magnitude is calculated. The frequency of excursion describes the number of times the contaminant may be present above the magnitude over the specified period (duration). A criterion is derived such that the combination of magnitude, duration, and frequency protects the designated use (e.g., primary contact recreation). Alternative criteria should include the same basic elements as the national RWQC.

In the 2012 RWQC, the EPA recommends expressing the criteria magnitude as a GM[12] value corresponding to the 50th percentile of the water quality distribution and an STV corresponding to the 90th percentile of the same water quality distribution. For duration and frequency, the EPA recommends that the waterbody GM not be greater than the selected GM magnitude in any 30-day interval. There should not be greater than a 10% excursion frequency of the selected STV magnitude in the same 30-day interval. Calculating the STV requires having the logarithm (log) standard deviation (logSD)[13] of the data for the indicator in the waterbody.

Table 2-8. Comparison of estimated or measured ingestion volumes per recreational event for Dufour et al. (2017) and DeFlorio-Barker et al. (2017).

Study	Age group	Number of participants	Median (mL)	Mean (mL)	Upper 90th percentile (mL)
Dufour et al. (2017)	All ages	549	19	32 (ln mean −3.98; ln SD 1.43)[a]	77
DeFlorio-Barker et al. (2017)	All ages	68,685[b]	16	44	105

Notes: ln = natural logarithm

a. For the forward QMRA, the distribution for ingestion is incorporated into the calculations (Section 2.2.2.4.3). For the reverse QMRA, the point estimate is used (Section 2.2.2.4.5).

b. This value represents the number of participants in epidemiological studies conducted across 12 beach locations which did not measure or estimate ingestion for each participant. DeFlorio-Barker modeled the distribution of water ingested by the 549 participants from the Dufour et al. (2017) study for the participants in the epidemiological studies.

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[12] The GM can be defined as the average of the log values converted back to base 10. For microbial monitoring data, transform each value to the base 10 log, find the arithmetic mean of the $\log_{10}$ values, and convert the result back to base 10.

[13] The logSD is the SD of the base 10 logs of the data. The antilog of the logSD would be the geometric SD.

2.2.2.4 Approach for adjusting the EPA's 2012 RWQC recommended FIB values for nonhuman sources.

As shown in Figure 1-1, four main steps are involved in the approach for adjusting the EPA's recommended 2012 RWQC FIB values to account for predominantly nonhuman fecal sources affecting a waterbody (see Section 3). At each step, there are risk management decision points to address, including whether to proceed with the TSM or adopt the nationally recommended criteria. Figure 2-6 displays a flow diagram of the overall process discussed in this TSM, including the risk management questions, major decision points, data collection activities, and QMRA analyses.

Step 1 (Section 3.1) is to conduct a **sanitary survey** to gain an understanding of the sources of fecal contamination in the watershed.

Step 2 (Section 3.2) is to conduct a **water quality study** to better understand both the initial assumptions and the results of the sanitary survey. Collectively, Steps 1 and 2 comprise a sanitary characterization. The sanitary characterization is critical for identifying and describing the fecal sources affecting the waterbody and for deriving site-specific criteria and will inform multiple science and policy decisions that are made to identify the predominant fecal source, conduct the QMRA, interpret the results, and derive alternative WQC.

Importantly, Steps 1 and 2 will also provide the information needed to decide if the human proportion of the fecal contamination in the watershed is small enough to warrant continuing to evaluate site-specific criteria for the watershed. For example, based on the data collected in Steps 1 and 2, it might be decided not to proceed with this process at this location and find another more representative site or instead adopt the nationally recommended values. It might be prudent to consider whether other factors, such as public perception or the ability to ensure protection for downstream waters, would influence the desirability or acceptability of site-specific criteria.

Step 3 (Section 3.3) is to **conduct an HHRA** based on the information/data collated and collected to determine human health risks due to the fecal sources identified in Steps 1 and 2. This step describes two QMRA-based approaches for using literature-derived and site-collected data to calculate pathogen and FIB densities in water (Sections 3.3.1 and 3.3.2). In all cases, the QMRA documentation should be clear and transparent. Importantly, at the end of this step, a determination is made of whether alternative criteria (including a fecal source and water quality evaluation and the target illness rate) can be developed or whether the recreational use for the study location is protected by the nationally recommended water quality and associated illness rates.

Step 4 (Section 3.4) is to **calculate a GM, STV and BAV** reflecting the predominantly nonhuman fecal source loading and corresponding to the chosen target illness rate. Once the sanitary characterization (Steps 1 and 2 above) and the HHRA (Step 3 above) are completed, the QMRA output can be used to determine what level of indicator (i.e., criteria magnitude) is protective of the primary contact recreational use in the waterbody.

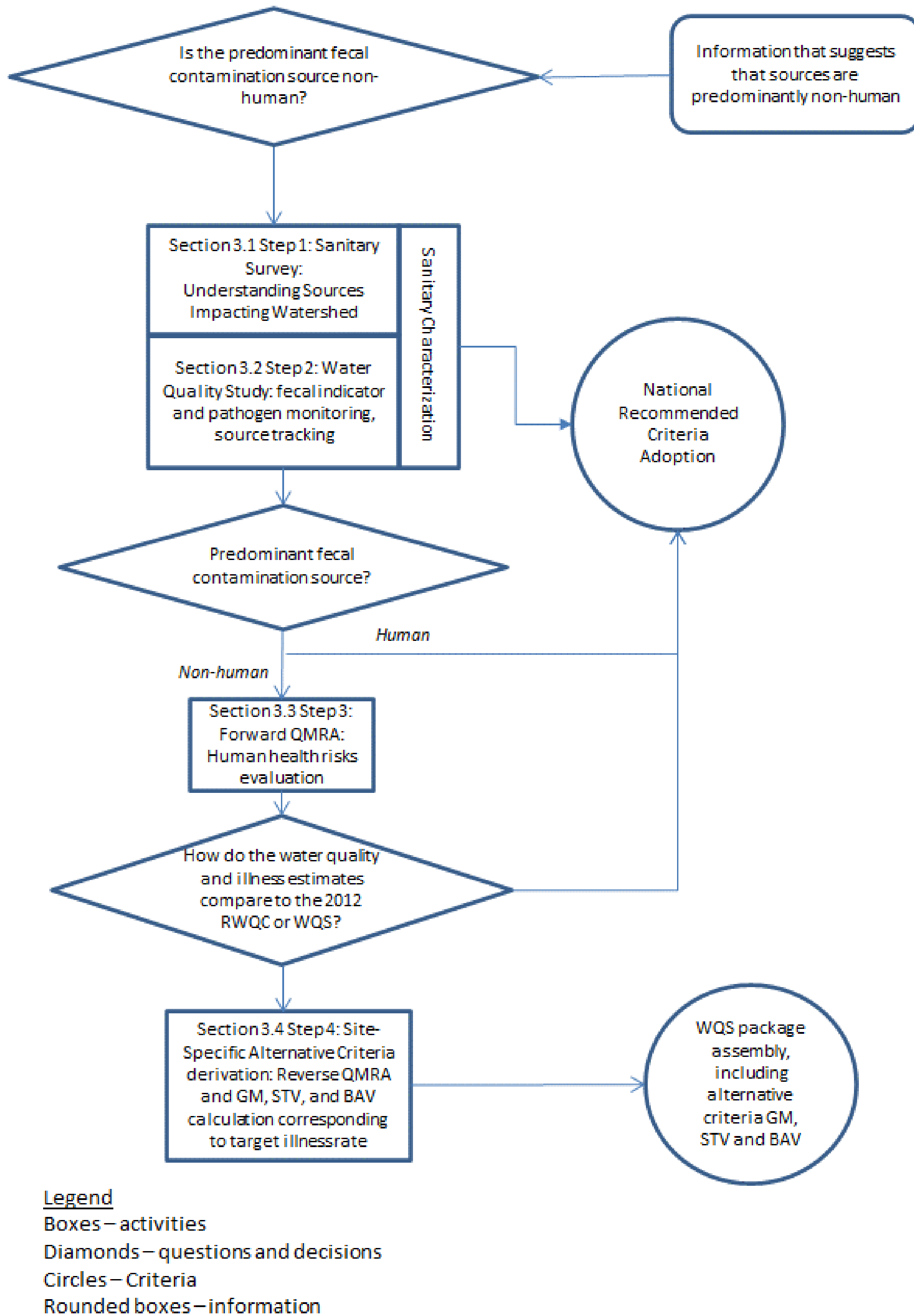


Figure 2-6. Overview diagram of the process discussed in this TSM for developing alternative criteria using QMRA for waters affected by predominantly nonhuman fecal sources.

The scientific literature describes two general types of QMRA modeling to evaluate potential human health risks that differ in the use of the input parameters. Deterministic modeling uses point estimates for each parameter. Stochastic modeling incorporates the distribution of values associated with each parameter. This TSM includes both types of QMRA modeling to address the risk management questions. For estimating the potential for gastrointestinal illness from primary contact recreational exposure, the suggested QMRA method for the human health risk evaluation is a stochastic Monte Carlo simulation-based model because it provides additional information on the potential ranges of the modeled outcomes. In this type of analysis, recreational events are treated probabilistically, and statistical distributions are used to characterize input parameters rather than point estimates. In contrast to a deterministic (point estimate-based) model, a stochastic QMRA model can include many thousands of iterative runs to yield a distribution of outcomes rather than a single-valued outcome. A deterministic QMRA approach is used in Step 4 to estimate the density of FIB corresponding to the target illness rate chosen.

Figure 2-7 displays the overall analytical plan for the QMRA conducted as part of this TSM. Pathogen densities in recreational waters can be considered for two main scenarios: (1) direct deposition and (2) indirect deposition. Direct deposition represents fresh feces from the fecal source affecting the waterbody with no environmental or treatment attenuation of pathogens considered before inputting the pathogen data into the health model. Indirect deposition represents a scenario where fecal sources may be land applied, as in the case of agronomic applications of manures, or from land-deposited fecal pats. The indirect deposition scenario includes consideration of physical and environmental fate and transport processes (e.g., runoff, sunlight, fecal age) that can result in microbial attenuation. The indirect scenario might be considered if pathogens are measured in land-applied feces and an estimate of fecal loading to a waterbody is evaluated. Measuring pathogens in feces can be helpful if levels in water are low and variable in occurrence (e.g., nonpoint sources dependent on wet weather-associated influx). See Soller et al. (2015) for an example of an indirect deposition scenario providing input into a QMRA. As discussed above in Sections 2.1.1.2 and 2.1.2.2, multiple potential fate and transport factors could be considered; generating data for all of them to provide input into this approach can be involved and resource-intensive. As discussed in Soller et al. (2015) the fate and transport construct can be simplified to one or a couple of important factors, such as stormwater runoff, coupled with health-protective assumptions, to provide informative illness estimates. Whichever deposition scenario is chosen, the user is encouraged to transparently document choices and provide substantiating data.

In this TSM, the health model can be run using three different approaches to address the different questions being addressed at each step. Discussion of the three main QMRA approaches can be found in Sections 2.2.2.4.3, 2.2.2.4.4, and 2.2.2.4.5. For example, if the starting point is the pathogen occurrence in recreational waters and the desired output is the total probability of illness, refer to Section 2.2.2.4.3 for the correct QMRA approach.

2.2.2.4.1 Summary of the EPA's Illustrative Scenario

The EPA has customized the QMRA framework in this TSM to specifically evaluate a recreational exposure scenario. The parameters needed for conducting QMRA, including exposure scenarios, are presented in this document to help simplify the overall approach and encourage consistency in the process and evaluation at the state level of potential alternative WQC reflecting nonhuman fecal source inputs. Therefore, some of the QMRA parameters are based on standard assumptions and

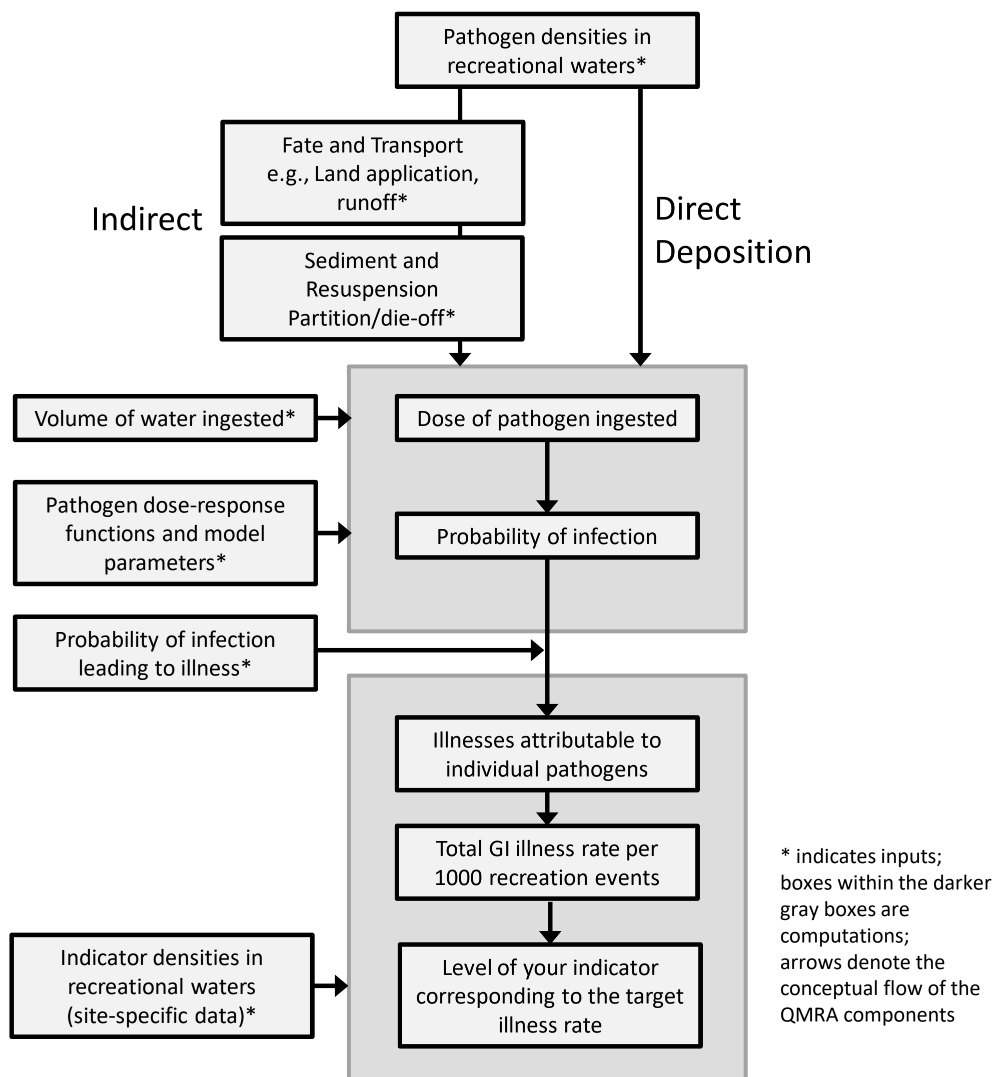


Figure 2-7. Conceptual diagram of the overall QMRA analytical approach described in this TSM.

others on site-specific information and data from Step 2 (Section 3.2). The EPA developed this approach and subjected its various components to independent scientific peer review to bolster the risk estimates' scientific defensibility and to encourage states to consider these tools within their respective WQS programs.

The EPA developed a specific exposure scenario (the "primary contact" scenario) for QMRAs addressing human exposures to recreational waters. The EPA purposefully constructed this exposure scenario to simplify the health modeling. For evaluating alternative criteria for waters contaminated by nonhuman fecal sources, the primary contact exposure scenario is as follows:

- The waterbody is predominantly impacted by a nonhuman source of contamination.
- The nonhuman source directly contributes[14] FIB or pathogens, or both, into the waterbody of interest.

[14] This is a health-protective assumption. Although the watershed may not have direct deposition, this assumption can still be chosen.

- FIB and reference pathogens occur in the fecal material of the source or in the nonfecal source at levels consistent with those reported in the peer-reviewed literature.
- Primary contact recreation occurs near and within a short period of time to the contamination (i.e., no die-off of either FIB or reference pathogens occurs).
- Primary contact recreation results in ingestion of water consistent with studies reported in the peer-reviewed literature (Dufour et al., 2017).

The EPA has previously published information to justify using the primary contact exposure scenario and this general approach (U.S. EPA, 2010a; Schoen and Ashbolt, 2010; Soller et al., 2010b, 2015). This exposure scenario is considered health-protective because it uses a hypothetical situation involving the direct deposition of fresh fecal material from a source to a waterbody in close proximity to recreators. Using these scenario assumptions, QMRA results indicate the mean probability of gastrointestinal illness associated with recreational exposure to waters impacted by fresh gull, chicken, or pig feces is substantially lower than in waters impacted by human sources (Soller et al., 2010b). The potential for gastrointestinal illness associated with recreational exposure to waters receiving direct deposition of fresh cattle feces is not substantially different from waters impacted by human sources. In waters affected by human fecal sources, risks were primarily associated with human enteric viruses, while in waters receiving bovine inputs, the pathogen profile associated with risk included a combination of zoonotic bacteria and protozoan pathogens (U.S EPA, 2010a; Soller et al., 2010a). This exposure scenario is also health-protective because pathogens are not attenuated via fate and transport or mobilization processes, nor does loading from additional sources occur that could increase indicator densities but without a commensurate increase in pathogens.

Refinement of the scenario to include land application of fecal material with mobilization of FIB and pathogens via undiluted runoff (introduction of fate and transport parameters), referred to as the “indirect deposition” scenario in Figure 2-7 above, can result in a reduction of estimates of potential of illness for all nonhuman sources, including cattle sources (U.S. EPA 2010a; Soller et al., 2015). These analyses demonstrate that the nature and magnitude of fecal contamination help to define the potential for human health impacts. Given the variability and uncertainty associated with the input parameters, the EPA decided to use this relatively straightforward and specific exposure scenario in its approach and highlight where additional fate and transport information could be included. This parsimonious approach can be extended to include more complicated or sophisticated fate and transport modeling, if desired, for specific waterbody conditions.

2.2.2.4.2 Sanitary Characterization

A central input into the QMRA-based framework discussed in this TSM is the need to identify and characterize the source(s) of fecal contamination affecting the waterbodies of interest. Different fecal sources (e.g., human versus nonhuman) can contribute different enteric pathogen profiles to fecal contamination, which can result in different levels of potential risks for recreators exposed to waters affected by different sources of contamination. The risk differential can manifest as different FIB values associated with the two target illness rates discussed in the RWQC. Nonfecal sources of *E. coli* and enterococci, such as sands and soils, algal mats and plant surfaces, and biofilms, also exist. Although an increase in illness has been demonstrated to be associated with sand exposure (Heaney et al., 2009), FIB enumerated from the beach sand have not been demonstrated to be predictive of the potential for human health effects (Halliday and Gast, 2011; Viau et al., 2011; Shibata and Solo-Gabriele, 2012).

Characterizing fecal source inputs will help to delineate the potential risks from nonhuman fecal sources relative to our understanding of the risks associated with exposure to human fecal contamination.

A key decision point in the overall framework discussed in this TSM is evaluating multiple lines of evidence to demonstrate that predominantly nonhuman sources of FIB influence the waterbody. Due to the importance of fecal source identification in determining fecal loading to a waterbody, the EPA has developed a "sanitary characterization" approach consisting of a two-step process for collating existing information on the potential fecal sources impacting the selected waterbody, and then collecting water quality data to substantiate the existence of nonhuman fecal sources and the absence of substantial human fecal inputs, such as wastewater effluent. Collecting data under different conditions (e.g., dry and wet weather periods) to understand contamination dynamics may be needed to fully characterize potential conditions and risks. The epidemiological data used to inform the 2012 RWQC recommendations were conducted in dry weather conditions. Wet weather scenarios can result in different indicator and health relationships (Arnold et al., 2017; Soller et al., 2017). It is critical to this QMRA framework that due diligence is used to identify and characterize the fecal source(s) affecting the waterbodies used to support adjusting the 2012 RWQC to account for nonhuman fecal sources.

Consider all evidence the sanitary survey and the water quality study provide. If a waterbody is in a more urbanized watershed, more evidence to demonstrate nonhuman fecal sources predominate might be needed because such waterbodies can be more susceptible to human fecal contamination and other anthropogenic inputs. Identifying and delineating human fecal contamination from nonfecal anthropogenic and nonanthropogenic inputs (e.g., FIB associated with algal mats and biofilm contributions of FIB) in urbanized watersheds can be challenging (Byappanahalli et al., 2003; Skinner et al., 2010; Piggot et al., 2012). Alternatively, less evidence might be needed for a remote location lacking significant surrounding urbanization, point sources, or both, to demonstrate adequately that human fecal contamination is low. For remote and pristine waterbodies, the initial sanitary survey might be sufficient evidence to demonstrate that nonhuman fecal sources are predominant. Decision-makers should also consider the "representativeness" of the waterbody studied if there is interest in generalizing these findings to other waterbodies with nonhuman fecal inputs.

2.2.2.4.2.1 Sanitary Survey

The first step in the sanitary characterization is a sanitary survey. A sanitary survey is a method of investigating the sources of fecal contamination to a waterbody (U.S. EPA, 2022). Although the literature includes many examples of sanitary surveys, including approaches used for drinking water source waters, shellfish, and watershed protection, the EPA has developed a sanitary survey form specific for use in the QMRA framework discussed in this TSM (see Appendix B for a QMRA sanitary survey form). The form found in Appendix B was based on the routine and annual beach sanitary surveys the EPA published previously. Additionally, the EPA has made available sanitary survey tools for fresh and marine recreational waters, including an application for Apple and Android operating systems (U.S. EPA, 2022). The information identified and collated as a part of this survey activity will help decide whether to proceed with the next step in the sanitary characterization or halt the process. This decision can involve both a science and a policy component, which can be transparently discussed as part of the documentation.

2.2.2.4.2.2 Water Quality Study

The second step of the sanitary characterization is to collect water quality information from the selected waterbody and watershed to substantiate the sanitary survey conclusions. The types of information included in a water quality study (see Section 3, Step 2) and the level of evidence needed could depend on local conditions. A robust understanding of the occurrence and prevalence of FIB and the types and loading of pathogens associated with the fecal sources in the watershed should be generated in the water quality study. Monitoring approaches should reflect conditions that result in fecal contamination. For example, if animal fecal contamination could occur from runoff of manures applied to fields, then monitoring following wet weather events is justified. Pre-existing monitoring data can be considered in this context as well.

Appendix C presents an example of a SAP for conducting a water quality study. This example includes a range of information that can be collected, and not all aspects need to be presented in the water quality study. A water quality study should be representative of the meteorological and hydrological conditions in the selected watershed. The two main purposes of this additional information are to develop scientifically defensible evidence that predominantly nonhuman fecal sources impact the waterbody and to supply the information necessary to estimate potential human health effects in the QMRA.

As part of the water quality study, it is recommended that microbial source identification and tracking (MST) techniques be incorporated to help confirm that the fecal sources that were identified in the sanitary survey are affecting the waterbody. The application of MST can help identify sources that the survey may have missed. Use of the microbial genetic source marker methods can also be helpful in understanding the dynamics of fecal contamination (i.e., sources affecting a waterbody under baseflow or wet weather conditions) and BMPs aimed at reducing contamination (Bushon et al., 2017; Li et al., 2019, 2021; Nevers et al., 2022; Shrestha et al., 2020). Characterized nonhuman microbial genetic source markers include bovine/ruminant (Shanks et al., 2008; Mieszkin et al., 2010; Raith et al., 2013; Xue and Feng, 2019), canine (Ervin et al., 2014; Green et al., 2014), avian/gulls/waterfowl (Green et al., 2012; McMinn et al., 2019), and swine (Mieszkin et al., 2009; Fan et al., 2017). Forty-one source markers have been evaluated for selectivity and specificity in a multi-laboratory trial (Boehm et al., 2013). The literature also includes several examples of the derivation of RBTs of selected MST markers (Boehm et al., 2015, 2018; Brown et al., 2017a; Ahmed et al., 2018; Schoen et al., 2020; Goh et al., 2021). Multiple human fecal source markers are available that can help confirm the presence of human fecal sources, such as sewage and effluent, and help evaluate if human fecal contamination predominates in the waterbody (Cao et al., 2018; Stachler et al., 2018; Schoen et al., 2020). The EPA has published draft Methods 1696 and 1697 for the characterization of human fecal contamination in water by HF183 and HumM2, respectively, via qPCR (U.S. EPA, 2019b,c). Recently, the EPA partnered with the National Institutes of Standards and Technology (NIST) to develop a high-quality standard control material (NIST SRM 2917) for use in MST qPCR methods (Willis et al., 2022). Choosing a well-characterized source marker can provide robust information on fecal sources in a water quality study and clear substantiation for decision points discussed in this TSM.

2.2.2.4.3 *"Forward" QMRA modeling*

A forward QMRA is an application of the health model using pathogen occurrence data as an input to calculate an estimate of the probability of illness. Forward QMRA includes the water quality information about the fecal source(s) and any monitoring data collected in the sanitary characterization. The resulting output of the analysis will provide a quantitative estimate of human health risks from recreational exposure to the waterbody of interest and can be used to compare with a target illness rate. The suggested QMRA method for the human health risk evaluation is a stochastic Monte Carlo simulation-based model. In this type of analysis, recreational events are treated probabilistically, and statistical distributions are used to characterize input parameters rather than point estimates. Figure 2-8 displays the overall process for a forward QMRA.

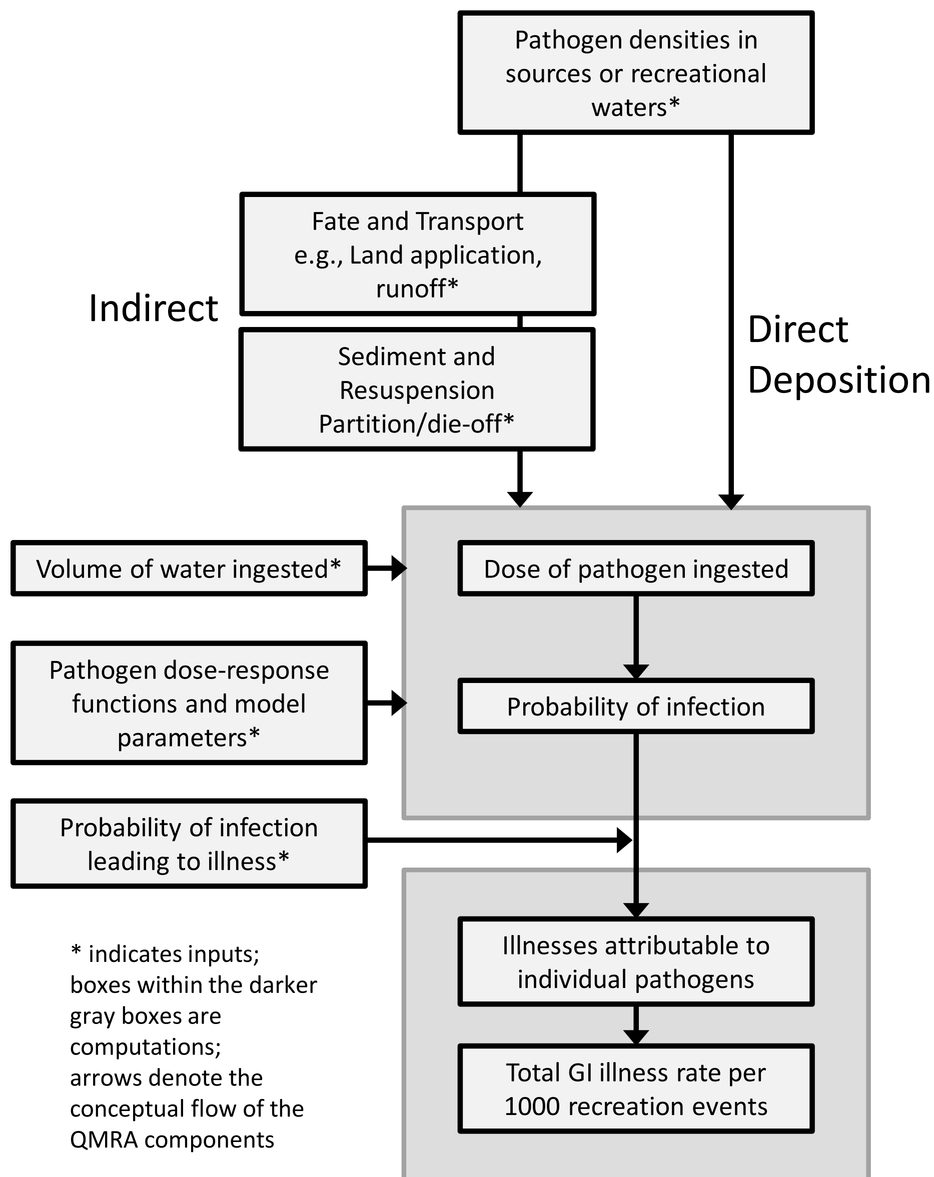


Figure 2-8. Analysis plan for a forward QMRA.

Forward QMRAs conducted in Step 3 (Section 3.3) bring together exposure and human health data in a logical way to estimate a predicted level of illness. As Figure 2-8 shows, reference pathogen densities are inputs to the human health risk evaluation. This TSM discusses two approaches to estimate the reference pathogen densities in Section 3.3.

- Approach 1: Estimate the waterbody pathogen density for each reference pathogen based on the reference pathogen data in the waterbody gathered during Step 2.
- Approach 2: Estimate the waterbody pathogen density for each reference pathogen from the FIB density in the waterbody, FIB density in the source, and reference pathogen density in the source following the methods of Schoen and Ashbolt (2010) and Soller et al. (2010b).

Both Approaches 1 and 2 provide valuable information for understanding the potential human health risks associated with recreational activities in the waterbody. Users of this TSM will need to do both approaches because they provide complementary perspectives. For example, the utility of Approach 1 is limited in cases where the enumeration of low levels of pathogens in ambient waters is limited by assay limits of detection (and quantification). A nondetection or nonquantified value in monitoring can mean that the pathogen is present at levels lower than the LOD or quantification of the enumeration method, not that the pathogen is absent. See Appendix C for a discussion of a sampling and analysis approach used at Boquerón Beach utilizing both quantitative enumeration methods and PCR-based presence-absence tests for specific pathogens. PCR-based methods can be more sensitive than culture-based methods, particularly at low levels of the target organism. However, they also may detect pathogens that are noninfectious or nonviable. Because pathogen occurrence in recreational waters varies temporally and spatially, monitoring results might not necessarily be representative of pathogen levels in the waterbody. Therefore, any subsequent risk analysis relying solely on these results (from Approach 1) might be misleading. In contrast, the direct deposition of fecal material (as in Approach 2), which does not account for pathogen or FIB decay, is expected to provide QMRA results at the higher end of the potential risk estimate. Taken together, QMRA results based on data from both Approaches 1 and 2 provide valuable insight for understanding the potential human health risks in the watershed.

Forward QMRA Approach 1: Use of data collected from water-quality monitoring

Approach 1 estimates the probability of gastrointestinal illness from primary contact recreational activities based on reference pathogen data sampled and measured in the waterbody during Step 2. Calculating the probability of illness by the method of forward QMRA involves computing the dose of each reference pathogen ingested during a recreational event, the probability of illnesses from each reference pathogen, and the combined total probability of illness from all reference pathogens using Equations 1A–1D.

$$\mu_{rp} = V \times C_{rp}^{M} \qquad \text{[Eq. 1A]}$$

where:

μ_{rp} is the dose ingested of reference pathogen *rp* (number of pathogens).

V is the volume of water ingested during the primary recreation activity (L; baseline assumption = 0.019 L).

C_{rp}^{M} is the measured density of reference pathogen *rp* in the waterbody (from site-specific data in units of pathogens/L).

$$P_{inf}^{rp} = f_{d-r}^{rp}\,(\mu_{rp}) \qquad \text{[Eq. 1B]}$$

where:

P_{inf}^{rp} is the probability of infection from reference pathogen *rp* (unitless).

$f_{d-r}^{rp}\,(\mu_{rp})$ is the mathematical dose-response function for reference pathogen *rp* (presented in Table 2-5) which estimates the probability of infection at a given dose (unitless).

$$P_{ill}^{rp} = p_{ill|inf}^{rp} \times P_{inf}^{rp} \qquad \text{[Eq. 1C]}$$

where:

P_{ill}^{rp} is the probability of illness from reference pathogen *rp* (unitless).

$p_{ill|inf}^{rp}$ is the proportion of individuals infected with reference pathogen *rp* who experience illness (unitless; presented in Table 2-6).

$$P_{ill} = 1 - \prod_{rp}(1 - P_{ill}^{rp}) \qquad \text{[Eq. 1D]}$$

where:

P_{ill} is the probability of illness from primary contact recreational activities in the waterbody accounting for the effects of all reference pathogens (unitless; assumes the independence of each reference pathogen).

Implementing equations 1A–1D using a single measurement of pathogen density will result in a point estimate of the probability of illness. Obtaining a statistical distribution of the probability of illness requires multiple measurements of pathogen density in the waterbody. These measurements can be used to conduct a Monte Carlo simulation, which produces a statistical distribution of the probability of illness. One method of using the pathogen density measurements in a Monte Carlo simulation is to randomly draw a single value of pathogen density from the range of measured values and estimate the probability of illness using this value and Equations 1A–1D above. Repeating this process thousands of times while replacing the drawn measurement value during each repetition generates a distribution of the probability of illness. An alternative method of using the measured pathogen densities in a Monte Carlo simulation is to first fit a statistical distribution to the measured density values using standard statistical techniques, randomly draw thousands of times from the fitted distribution of densities and process the randomly drawn density value each time through Equations 1A–1D. This procedure also will result in a statistical distribution for the probability of illness. In addition to randomly drawing pathogen densities, the Monte Carlo simulation can also randomly draw from statistical distributions for the volume of water ingested and the various dose-response parameters.

This approach is still valid if reported pathogen densities are below the LOQ—or LOD—or if the dataset contains many nondetects. It is not unexpected for reported reference pathogen densities to be consistently below detectable limits, partly due to the variability in prevalence in the hosts and occurrence in environmental matrices. However, there are approaches that can be used to address nondetects. One approach is to assume that the average organism density is at the respective

detection limit of the enumeration method being used (Soller et al., 2016). Because the actual pathogen density is not greater than the detection limit in this case, this approach can provide a reasonable upper-bound estimate of pathogen density. Other approaches for addressing nondetects and left-censored data can be found in the literature (Helsel, 2012; Schmidt et al., 2013; Canales et al., 2018).

Appendix E contains annotated computer code in R that can be used to implement the suggested stochastic QMRA approach.[15]

Forward QMRA Approach 2: Using a combination of collected and literature-based data
In Approach 2, the waterbody reference pathogen densities are estimated based on the measured FIB[16] density in the waterbody, FIB density in the source, and reference pathogen density in the source following the methods of Schoen and Ashbolt (2010) and Soller et al. (2010b). This process involves sequentially estimating:

1. The density of each reference pathogen in the waterbody deriving from each source.
2. The ingested dose of each reference pathogen attributable to each source.
3. The probability of illness from each reference pathogen attributable to each source.
4. The combined probability of illness counting the contribution of all reference pathogens for each source.
5. The total probability of illness combining the contribution of all pathogens from all sources in the waterbody.

Equations 2A–2F summarize these steps.

$$C_{rp}^{S} = p_{FIB}^{S} \times C_{FIB} \times \frac{R_{rp}^{S}}{R_{FIB}^{S}} \qquad \text{[Eq. 2A]}$$

where:

C_{rp}^{S} is the estimated density of the reference pathogen (number of pathogens or genomes/L) in the waterbody deriving from Source *S*.

p_{FIB}^{S} is the proportion of FIB in the waterbody attributable to Source *S* (unitless; from the site-specific information).

C_{FIB} is the waterbody density of FIB measured using a culture-based method (CFU/L) (from the site-specific data). Note to users: For example, enterococci measurements in water are frequently reported in units of CFU/100 mL. However, Equation 2A requires that the measurements be converted into units of CFU/L.

R_{FIB}^{S} is the density of FIB using a culture-based method (CFU/L or CFU/g) in Source *S* (Table 2-7 or the source).

[15] R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.
[16] In the examples in this TSM, the EPA used enterococci. *E. coli* can also be used.

R_{rp}^{S} is the density of the reference pathogen (number of pathogens or genomes/L or number of pathogens or genomes/g) in Source *S* (Table 2-2 or the source).

$$\mu_{rp}^{S} = C_{rp}^{S} \times p_{rp}^{S} \times I_{rp}^{S} \times V \qquad \text{[Eq. 2B]}$$

where:

μ_{rp}^{S} is the estimated dose of human infectious reference pathogens (number of pathogens or genomes) attributable to the source.

p_{rp}^{S} is the fraction of human infectious pathogenic strains from Source *S* (unitless; presented in Table 2-4).

I_{rp}^{S} is the fraction prevalence of infection in the Source *S* (unitless; presented in Table 2-3).

V is the volume of water ingested during the recreational event (L; baseline assumption = 0.019 L).

$$P_{inf}^{rp,S} = f_{d-r}^{rp}\,(\mu_{rp}^{S}) \qquad \text{[Eq. 2C]}$$

where:

$P_{inf}^{rp,S}$ is the probability of infection from reference pathogen *rp* originating from source *S* (unitless).

$f_{d-r}^{rp}\,(\mu_{rp})$ is the mathematical dose-response function for reference pathogen *rp* (presented in Table 2-5), which estimates the probability of infection at a given dose (unitless).

$$P_{ill}^{rp,S} = p_{ill|inf}^{rp} \times P_{inf}^{rp,S} \qquad \text{[Eq. 2D]}$$

where:

$P_{ill}^{rp,S}$ is the probability of illness from a specific reference pathogen *rp* originating from Source *S* (unitless).

$p_{ill|inf}^{rp}$ is the proportion of individuals infected with reference pathogen *rp* who experience illness (unitless; presented in Table 2-6).

$$P_{ill}^{S} = 1 - \prod_{rp}(1 - P_{ill}^{rp,S}) \qquad \text{[Eq. 2E]}$$

where:

P_{ill}^{S} is the estimated probability of illness from a specific source of contamination *S* (unitless; assumes the independence of each reference pathogen in Source S).

$$P_{ill} = 1 - \prod_{S}(1 - P_{ill}^{S}) \qquad \text{[Eq. 2F]}$$

where:

P_{ill} is the estimated total probability of illness from all sources of contamination and all pathogens in the waterbody (unitless; assumes the independence of each contamination source).

As discussed in Approach 1, creating a distribution of the estimated probability of illness is possible using Monte Carlo simulation. Creating a distribution requires random drawing with replacement multiple times from the FIB distribution and, potentially, random drawing from the statistical distributions of the volume of water ingested and the dose-response parameters.

Table 2-9 summarizes the definitions of the variables in the above equations, provides the notation for each variable, and indicates where the data for the variable can be located or indicates that the data are from the water quality study. Appendix F contains annotated computer code in R that is publicly available and can be used to implement Approach 2.

Table 2-9. Approaches 1 and 2: definitions and sources of variables used in equations.

Variable	Definition	Data source
C_{rp}^{M}	Measured density of reference pathogen in waterbody (pathogens per L)	Site-specific reference pathogen data
C_{rp}^{S}	Estimated density of reference pathogen in waterbody derived from Source S (pathogens per L)	Calculated based on Equation 2A
C_{FIB}	Waterbody density of FIB using a culture method (CFU per L)	Site-specific FIB (enterococci or E. coli) data (GM calculated for the waterbody in Step 2)
R_{FIB}^{S}	Density of FIB using a culture method (CFU/L or CFU/g) in the source	Table 2-7 data are from the peer-reviewed scientific literature. For other fecal sources, directly gather enterococci density in the sources at the site or find published information.
R_{rp}^{S}	Density of each reference pathogen from Source S (number of pathogens or genomes/L or number of pathogens or genomes/g)	Table 2-2 data are from the peer-reviewed scientific literature. For other fecal sources, directly gather pathogen densities in the sources at the site or find published information.
μ_{rp}^{S}	Estimated dose of reference pathogen derived from Source S (number of pathogens ingested)	Calculated based on Equation 1A or 2B
p_{FIB}^{S}	Proportion of FIB deriving from source S (unitless)	Site-specific information
p_{rp}^{S}	Fraction of human infectious pathogenic strains (unitless)	Table 2-4
I_{rp}^{S}	Prevalence of infection in the nonhuman source (unitless)	Table 2-3
V	Volume of water ingested (L)	Described in the text (point estimate of 0.019 L per person per primary contact recreation event)
P_{inf}^{rp}	Probability of infection from reference pathogen rp (unitless)	Calculated based on Equation 1B
$P_{inf}^{rp,S}$	Probability of infection from reference pathogen rp originating from Source S (unitless)	Calculated based on Equation 2C
P_{ill}^{rp}	Probability of illness from reference pathogen rp (unitless)	Calculated based on Equation 1C
$P_{ill}^{rp,S}$	Probability of illness from a specific reference pathogen rp from Source S (unitless)	Calculated based on Equation 2D
P_{ill}^{S}	Probability of illness from Source S from all pathogens (unitless)	Calculated based on Equation 2E
P_{ill}	Probability of illness from all sources (unitless)	Calculated based on Equation 1D or 2F

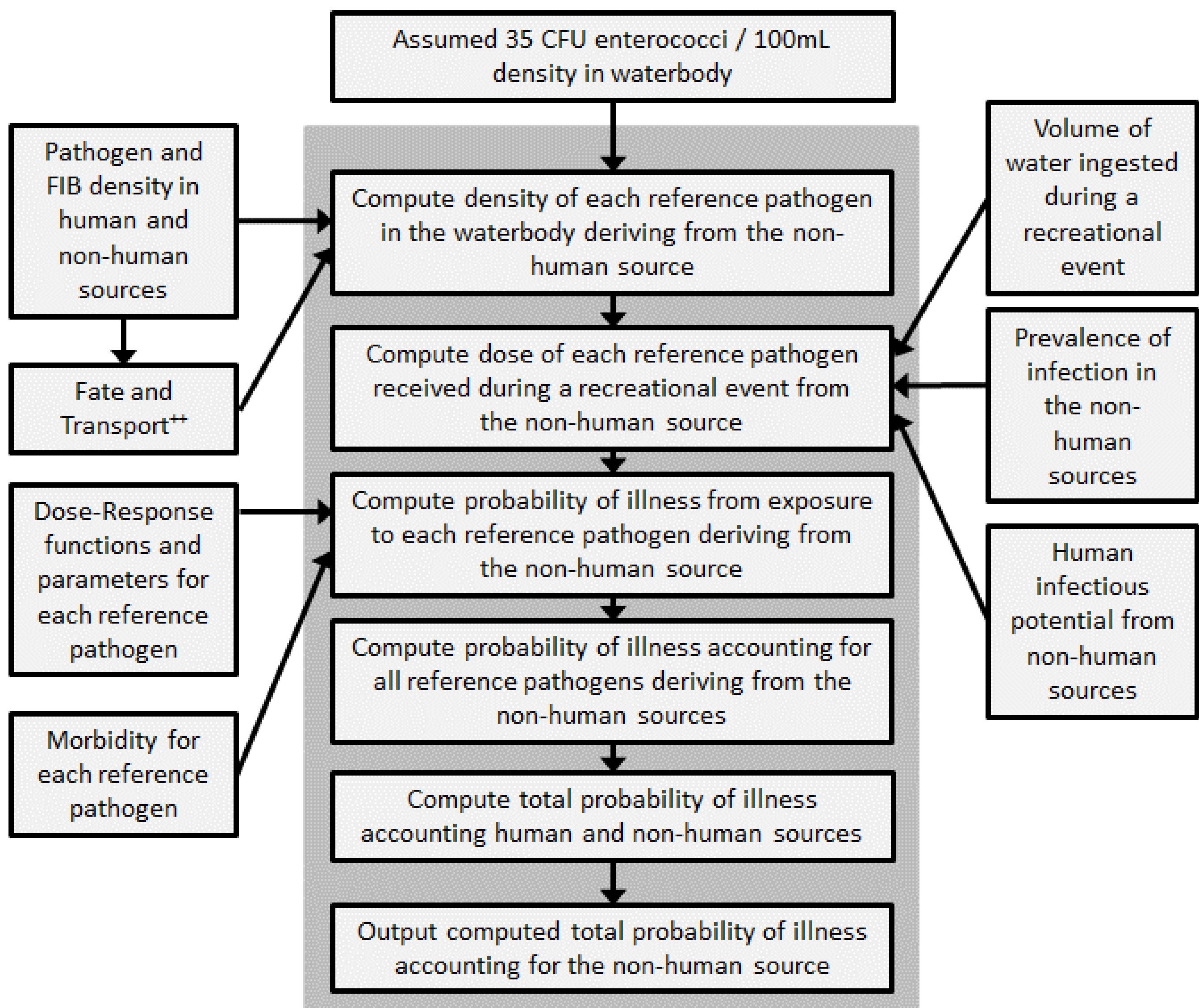
2.2.2.4.4 "Relative" QMRA modeling

A relative QMRA complements a forward QMRA by normalizing risks from specific fecal sources to a specified FIB density (Soller et al., 2015). A relative QMRA is an application of the health model in a scenario where both the monitored FIB and estimated risk from the forward QMRA are elevated above the recommended water quality value and target illness rate, respectively. If the sanitary characterization indicates that nonhuman sources predominate, then a relative QMRA can be used to understand what the estimated target illness rate would be in the waterbody when it is meeting the EPA's recommended RWQC FIB levels. The level and types of pathogens determine the risk due to fecal contamination, but WQS are based on the level of FIB; therefore, to compare the water at the study site to the EPA's recommended water quality values and target illness rate, there is a need to normalize the risk estimates to the EPA's recommended level of FIB. The analytical approach displayed in Figure 2-9 shows how a QMRA can be designed to answer the question: What would the predicted illness level be if the waterbody water quality was at 35 enterococci CFU per 100 mL? Conducting a relative QMRA can assist users in interpreting the forward QMRA results and provide additional substantiation for alternative WQC developed in Step 4 (Section 3.4).

Comparing Figure 2-9 to the forward QMRA displayed in Figure 2-8, the input data differ from those used in the forward QMRA. For the relative QMRA, a specific level of FIB is assumed in the waterbody—in this case, the current RWQC levels for enterococci or *E. coli*. These FIB levels are used in conjunction with the FIB and reference pathogen levels in the land-applied material, the fraction of human-infectious pathogenic strains in each fecal source of interest, the prevalence of infection in the nonhuman source (proportion of animals shedding the pathogen), the proportions of FIB and reference pathogens that mobilize during a rain event, and the volume of water ingested (Schoen and Ashbolt, 2010; Soller et al., 2010b). The relative QMRA model output is the probability of infection and illness associated with exposure to water during recreation for each source of interest referenced to the chosen level of FIB. Once the data are normalized to the EPA's recommended water quality value, two outcomes are possible. If the mean of the predicted illness level is below the target illness rate, then continue to Step 4. If the mean predicted illness level is above the target illness rate, then the nationally recommended criteria apply and do not proceed further with this TSM at this study location. See further discussion on interpreting results of the forward QMRA for cases where predicted illness levels are higher than the illness level benchmark in Section 3.3.3.

2.2.2.4.5 "Reverse" QMRA modeling

Commonly, QMRAs are conducted using microbial density as an input, and the output is the estimated risk level as was done for Approaches 1 and 2 in Step 3. In contrast, a "reverse" QMRA is when the input is the risk level, and the corresponding microbial density is calculated (Figure 2-10). Reverse QMRA is used in Step 4 (Section 3.4) to solve for FIB density rather than risk level. Conducting a reverse QMRA in Step 4 results in the calculation of the alternative FIB GM value associated with the predominant source of nonhuman fecal contamination affecting the waterbody. The same QMRA parameters are used as described above in Step 3: (1) the volume of water ingested during swimming; (2) the density of pathogens in the specific sources; (3) the mathematical dose-response relationships and the parameter values for each reference pathogen; and (4) the conditional probability of illness given infection for different pathogens. In contrast to the stochastic approach used in the forward QMRA, the reverse QMRA uses a deterministic approach because a stochastic reverse QMRA can be highly computationally intensive, and the output desired is a single value (i.e., a single FIB GM) instead



++ Fate and Transport modeling is optional
Boxes outside of the darker gray box are model inputs and boxes within the darker gray box are computations.
Arrows denote the conceptual flow of the relative QMRA model

Figure 2-9. Analytical approach for “relative” QMRA anchored at a specific FIB level.

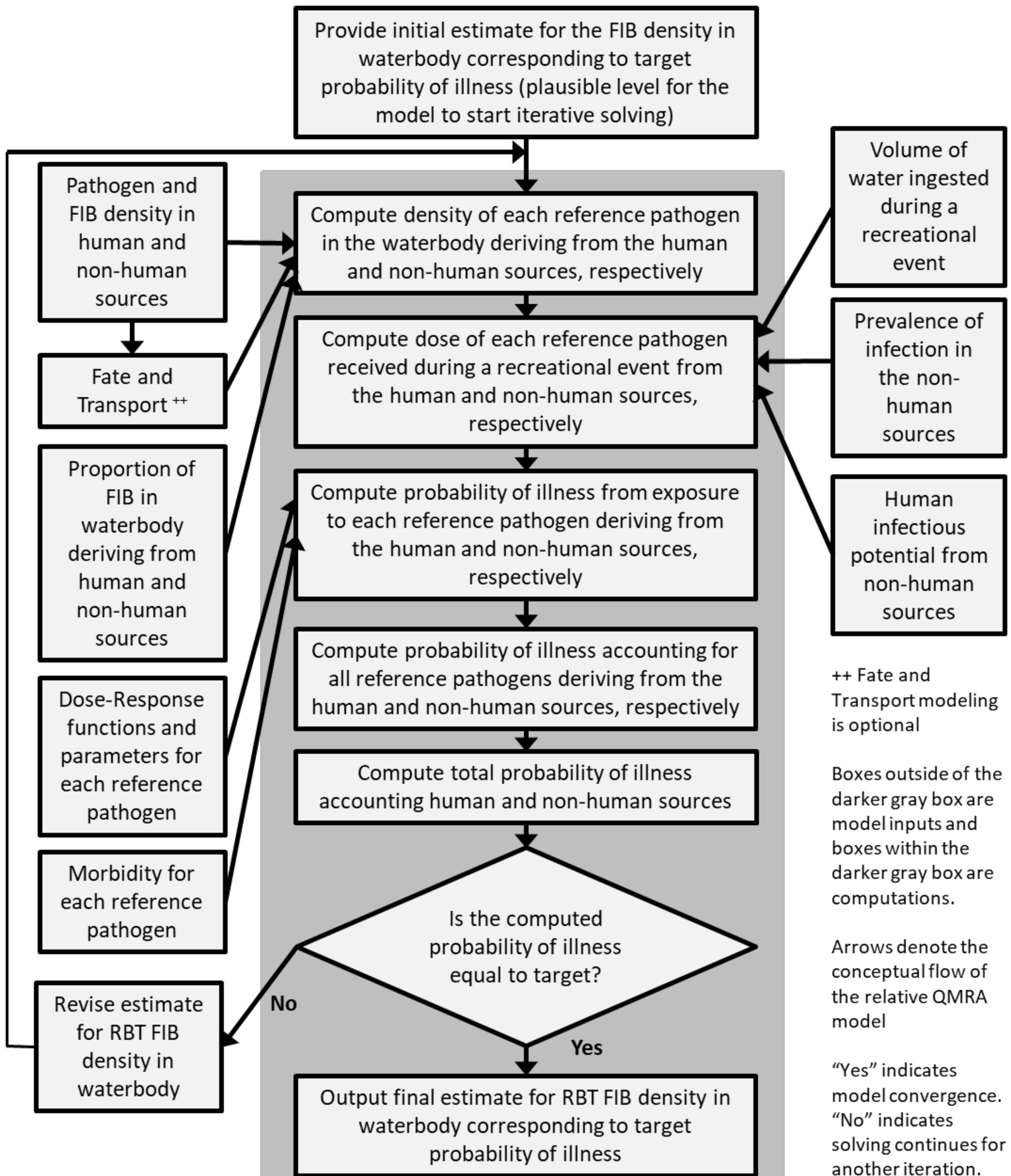


Figure 2-10. Analytical approach for reverse QMRA.

of a range. These QMRA calculations can estimate the culturable enterococci or *E. coli* level for various mixtures of human and nonhuman contamination. A case study example is provided in Step 4 to illustrate the process.

The EPA has conducted and documented QMRAs for waters affected by human, cattle, pig, chicken, gull, or nonpathogenic fecal sources (Schoen and Ashbolt, 2010; Soller et al., 2010a,b, 2014, 2015; Schoen et al., 2011). An important result noted in the QMRAs evaluating mixed fecal sources was that the risks are determined predominantly by the proportion of the contamination source with the greatest ability to cause human infection, not necessarily the source that contributes the greatest number of FIB (Schoen et al., 2011; Soller et al., 2014). When human sources are a component of the mixed fecal loading, the viral pathogens in human waste pose the highest risk of gastrointestinal illness to recreators. Soller et al. (2014) demonstrated that the estimated risks of gastrointestinal illness from recreational exposures to fecal mixtures were up to 50% lower for mixtures with an approximately 30% human component compared to fecal loadings of 100% human as measured by enterococci. If there was gull, pig, or chicken fecal contamination and a low human contribution (i.e., less than the approximate 30% contribution), reverse QMRA-predicted enterococci GMs were substantially higher than the 2012 RWQC water quality recommendations but based on the same level of health protection.

For reverse QMRA, estimating the FIB levels for recent human and animal contamination mixtures that correspond to a specific target illness rate requires conducting a set of analyses, which essentially perform the inverse of the Step 3 QMRA analyses described above for a specific level of predicted illness. The steps below (which correspond to the example Python code Appendix G presents) are the same as those used in Soller et al. (2010b, 2015).

This QMRA initially uses a preliminary value for the FIB density (enterococci in this example) in the waterbody to compute the density of each reference pathogen attributable to each source as described in Equations 3A and 3B. Derive this FIB density via numerical iteration as described below.

$$C_{rp}^{NHS} = p_{FIB}^{NHS} \times C_{FIB} \times \frac{R_{rp}^{NHS}}{R_{FIB}^{NHS}} \quad \text{[Eq. 3A]}$$

where:

C_{rp}^{NHS} is the estimated density of the reference pathogen (number of pathogens or genomes/L) in the waterbody deriving from the nonhuman source *NHS*.

p_{FIB}^{NHS} is the proportion of FIB in the waterbody that is attributable to the nonhuman source *NHS* (unitless; from site-specific information).

C_{FIB} is an initial estimate for FIB density (CFU/L) in the waterbody that is assumed to correspond to the required target probability of illness (this estimate is iteratively updated).

R_{rp}^{NHS} is the density of the reference pathogen (number of pathogens or genomes/L or number of pathogens or genomes/g) in the nonhuman source *NHS* (Table 2-5 or the source at the site).

R_{FIB}^{NHS} is the density of FIB (CFU/L or CFU/g) in the nonhuman source *NHS* (Table 2-7 or the source at the site).

$$C_{rp}^{HS} = p_{FIB}^{HS} \times C_{FIB} \times \frac{R_{rp}^{HS}}{R_{FIB}^{HS}} \quad \text{[Eq. 3B]}$$

where:

C_{rp}^{HS} is the estimated density of the reference pathogen (number of pathogens or genomes/L) in the waterbody deriving from the human source *HS*.

p_{FIB}^{HS} is the proportion of FIB in the waterbody that is attributable to the human source *HS,* which is equivalent to $(1 - \; p_{FIB}^{NHS})$ defined above (unitless).

C_{FIB} is an initial estimate for FIB density (CFU/L) in the waterbody that is assumed to correspond to the required target probability of illness (this estimate is iteratively updated).

R_{rp}^{HS} is the density of the reference pathogen (number of pathogens or genomes/L or number of pathogens or genomes/g) in the human source *HS*.

R_{FIB}^{HS} is the density of FIB (CFU/L or CFU/g) in the human source *HS*.

If the human source is a mixture of raw and secondary treated and disinfected WWTP effluent, compute the ratio of pathogens to FIB in the human source in Equation 3B above as follows (also see Soller et al., 2010b):

$$\frac{R_{rp}^{HS}}{R_{FIB}^{HS}} = 0.9889 \frac{R_{rp}^{raw}}{R_{FIB}^{raw}} + 0.0111 \frac{R_{rp}^{WWTP}}{R_{FIB}^{WWTP}} \quad \text{[Eq. 3C]}$$

where:

R_{FIB}^{raw} is the density of FIB (CFU/g) in raw human contamination (Table 2-7).

R_{rp}^{raw} is the density of the reference pathogen (number of pathogens or genomes/g) in raw human contamination (Table 2-5).

R_{FIB}^{WWTP} is the density of FIB (CFU/L) in the WWTP-treated human contamination (Table 2-7).

R_{rp}^{WWTP} is the density of the reference pathogen (number of pathogens or genomes/L) in WWTP-treated human contamination (Table 2-5).

Note that the constants in Equation 3C are derived by anchoring a probability of 36 NGI per 1,000 recreators[17] to an enterococci level of 35 CFU per 100 mL for an ingestion volume of 0.019 L (i.e., anchoring to the EPA's recommended target illness rate and corresponding water quality value). If the anchor levels are adjusted either for the illness rate or the culture-enumerated FIB, the constants in Equation 3C may also need to be adjusted.

Then, compute the dose of each reference pathogen attributable to each source per Equations 3D and 3E.

$$\mu_{rp}^{NHS} = V \times C_{rp}^{NHS} \times p_{rp}^{NHS} \times I_{rp}^{NHS} \quad \text{[Eq. 3D]}$$

[17] The recommended health goal associated with the national 2012 RWQC is 32 or 36 NGI per 1,000 primary contact recreation events. These two values are not significantly different.

$$\mu_{rp}^{HS} = V \times C_{rp}^{HS} \quad \text{[Eq. 3E]}$$

where:

> μ_{rp}^{NHS} is the estimated dose of human infectious reference pathogens (number of pathogens or genomes) attributable to the nonhuman source.
>
> *V* is the volume of water ingested during the recreational event (0.019 L; Table 2-8).
>
> p_{rp}^{NHS} is the fraction of human infectious pathogenic strains from the nonhuman source *NHS* (unitless; presented in Table 2-4).
>
> I_{rp}^{NHS} is the fraction prevalence of infection in the nonhuman source *NHS* (unitless; presented in Table 2-3).
>
> μ_{rp}^{HS} is the estimated dose of human infectious reference pathogens (number of pathogens or genomes) attributable to the human source.

Compute the probability of infection from exposure to each reference pathogen derived from each source per Equations 3F and 3G.

$$P_{inf}^{rp,NHS} = f_{d-r}^{rp}\,(\mu_{rp}^{NHS}) \quad \text{[Eq. 3F]}$$

$$P_{inf}^{rp,HS} = f_{d-r}^{rp}\,(\mu_{rp}^{HS}) \quad \text{[Eq. 3G]}$$

where:

> $P_{inf}^{rp,NHS}$ is the probability of infection from a specific reference pathogen *rp* originating from the nonhuman source *NHS* (unitless).
>
> $P_{inf}^{rp,HS}$ is the probability of infection from a specific reference pathogen *rp* originating from the human source *HS* (unitless).
>
> f_{d-r}^{rp} is the mathematical dose-response function for reference pathogen *rp* (presented in Table 2-5) that estimates the probability of infection at a given dose (unitless).

Compute the probability of illness from exposure to each reference pathogen derived from each source per Equations 3H and 3I.

$$P_{ill}^{rp,NHS} = p_{ill|inf}^{rp} \times P_{inf}^{rp,NHS} \quad \text{[Eq. 3H]}$$

$$P_{ill}^{rp,HS} = p_{ill|inf}^{rp} \times P_{inf}^{rp,HS} \quad \text{[Eq. 3I]}$$

where:

> $P_{ill}^{rp,NHS}$ is the probability of illness from a specific reference pathogen *rp* originating from the nonhuman source *NHS* (unitless).
>
> $P_{ill}^{rp,HS}$ is the probability of illness from a specific reference pathogen *rp* originating from the human source *HS* (unitless).

$p_{ill|inf}^{rp}$ is the proportion of individuals infected with reference pathogen *rp* who experience illness (unitless; presented in Table 2-6).

Compute the probability of illness from exposure to each source, accounting for the effect of all reference pathogens within that source, per Equations 3J and 3K.

$$P_{ill}^{NHS} = 1 - \prod_{rp}(1 - P_{ill}^{rp,NHS}) \quad \text{[Eq. 3J]}$$

$$P_{ill}^{HS} = 1 - \prod_{rp}(1 - P_{ill}^{rp,HS}) \quad \text{[Eq. 3K]}$$

where:

P_{ill}^{NHS} is the estimated probability of illness from the nonhuman source of contamination *NHS* (unitless; assumes the independence of each reference pathogen).

P_{ill}^{HS} is the estimated probability of illness from the human source of contamination *HS* (unitless; assumes the independence of each reference pathogen).

Compute the total probability of illness accounting for exposure to both the human and nonhuman sources using Equation 3L.

$$P_{ill} = 1 - \left(1 - P_{ill}^{NHS}\right) \times (1 - P_{ill}^{HS}) \quad \text{[Eq. 3L]}$$

where:

P_{ill} is the estimated total probability of illness accounting for exposure to both the nonhuman and human sources of contamination in the waterbody (unitless; assumes the independence of risk from human and nonhuman sources).

If the computed value of the total probability of illness in Equation 3L differs from the target probability of illness, the initial estimate for the FIB density in the waterbody must be iteratively adjusted until the computed value agrees with the target value of probability of illness. Usually, that can be readily accomplished using analytical software packages. The FIB density level in the final iteration of this procedure is the waterbody FIB density level that corresponds to the target probability of illness for the specified contamination mixture. Table 2-10 summarizes the variables in the above text and includes where the information for the variables can be found in the TSM. In this TSM example, enterococci was used as the FIB.

The values generated from modeling would apply if the sources affecting the waterbody were well understood and characterized. In addition, the land use patterns in the watershed also would need to be consistent with the conditions under which the water quality study was conducted (Step 2). Note that it is unlikely that any waterbody with a WWTP outfall would qualify as having a predominantly nonhuman source. Waterbodies potentially affected by sanitary infrastructure failures need to be evaluated carefully in this process by understanding under what conditions exfiltration from infrastructure, septage in poorly draining soils, and other potential human inputs may occur (e.g., rainfall-associated).

Table 2-10. Reverse QMRA: definitions and sources for variables in equations.

In-text variable	Definition	Data sources
C_{rp}^{NHS}	Estimated density of reference pathogen in waterbody derived from the nonhuman source NHS (pathogens per L)	Calculated based on equation 3A
C_{rp}^{HS}	Estimated density of reference pathogen in waterbody derived from the nonhuman source HS (pathogens per L)	Calculated based on equation 3B
C_{FIB}	Waterbody density of FIB based on a culture method (CFU/L)	Initial estimate that is iteratively updated
p_{FIB}^{NHS}	Proportion of FIB deriving from the nonhuman source NHS (unitless)	Site-specific information
p_{FIB}^{HS}	Proportion of FIB deriving from the human source HS (unitless)	Calculated based on $(1 - p_{FIB}^{NHS})$
R_{FIB}^{NHS}	Density of FIB (CFU/L or CFU/g) in the nonhuman source NHS	Table 2-7 data are from the peer-reviewed scientific literature. For other fecal sources, directly gather FIB densities in sources at the site or find published information.
R_{FIB}^{HS}	Density of FIB (CFU/L or CFU/g) in the human source HS	Calculated as ratio to pathogen density
R_{rp}^{NHS}	Density of each reference pathogen in the nonhuman source NHS (number of pathogens or genomes/g or number of pathogens or genomes/L)	Table 2-2 data are from the peer-reviewed scientific literature. For other fecal sources, directly gather reference pathogen density in sources at the site or find published information.
R_{rp}^{HS}	Density of each reference pathogen in the human source HS (number of pathogens or genomes/g or number of pathogens or genomes/L)	Calculated as ratio to FIB density
R_{FIB}^{raw}	Density of FIB (CFU/g) in raw human contamination	Table 2-7 data are from the peer-reviewed scientific literature. For other fecal sources, directly gather FIB density in sources at the site or find published information.
R_{FIB}^{WWTP}	Density of FIB (CFU/L) in WWTP-treated human contamination	Table 2-7 data are from the peer-reviewed scientific literature. For other fecal sources, directly gather FIB density in sources at the site or find published information.
R_{rp}^{raw}	Density of each reference pathogen in raw human contamination (number of pathogens or genomes/g)	Table 2-7 data are from the peer-reviewed scientific literature. For other fecal sources, directly gather reference pathogen density in sources at the site or find published information.
R_{WWTP}^{rp}	Density of reference pathogens in WWTP-treated human contamination (number of pathogens or genomes/L)	Table 2-7 data are from the peer-reviewed scientific literature. For other fecal sources, directly gather reference pathogen density in sources at the site or find published information.
μ_{rp}^{NHS}	Estimated dose of reference pathogen derived from nonhuman source NHS (number of pathogens)	Calculated based on equation 3D
μ_{rp}^{HS}	Estimated dose of reference pathogen derived from human source HS (number of pathogens)	Calculated based on equation 3E

In-text variable	Definition	Data sources
p_{rp}^{NHS}	Fraction of human infectious pathogenic strains of the reference pathogen in the nonhuman source (unitless)	Table 2-4
I_{rp}^{NHS}	Prevalence of infection in the nonhuman source for the reference pathogen (unitless)	Table 2-3
V	Volume of water ingested (L)	Described in the text (point estimate of 0.019 L per person per primary contact recreation event)
$P_{inf}^{rp,NHS}$	Probability of infection from a specific reference pathogen rp deriving from the nonhuman source NHS (unitless)	Calculated based on equation 3F
$P_{inf}^{rp,HS}$	Probability of infection from a specific reference pathogen rp deriving from the human source HS (unitless)	Calculated based on equation 3G
$P_{ill}^{rp,NHS}$	Probability of illness from a specific reference pathogen rp deriving from the nonhuman source NHS (unitless)	Calculated based on equation 3H
$P_{ill}^{rp,HS}$	Probability of illness from a specific reference pathogen rp deriving from the human source HS (unitless)	Calculated based on equation 3I
P_{ill}^{NHS}	Probability of illness from all pathogens in the nonhuman source NHS (unitless)	Calculated based on equation 3J
P_{ill}^{HS}	Probability of illness from all pathogens in the human source HS (unitless)	Calculated based on equation 3K
P_{ill}	Probability of illness from the human and nonhuman sources (unitless)	Calculated based on equation 3L

3.0 Site-Specific Alternative Criteria Development for Waters Predominantly Impacted by Nonhuman Fecal Contamination

This section discusses each step in developing alternative criteria by adjusting the EPA's recommendations for culturable *E. coli* and enterococci by accounting for the potential lower human health risk associated with exposure to primarily nonhuman fecal sources. The information is presented with a level of detail sufficient for a technical audience (i.e., those conducting evaluations to develop the criteria).

To initiate the data collection and analysis steps discussed in this section, users of this document will have completed the planning and scoping process described in Section 2 and have identified one or more potential study locations where evidence suggests nonhuman fecal sources are likely to predominate. The process described in this section incorporates the microbial and exposure parameters information presented in Section 2 and the user-supplied information into the QMRA-based framework pictured in Figure 2-6 (see Section 2.2.2.4). Steps 1–4 described in this section correspond to the four boxes in Figure 2-6. In each section, a more detailed figure for each box corresponding to Figure 2-6 is presented, decision points are identified, and an illustrative example of the process for each step is provided. Documenting the details in a transparent and clear way in supporting documentation will facilitate public comment by stakeholders and the EPA's evaluation of WQS submissions.

Feces-contaminated recreational waters can contain pathogens with the potential to cause human illness. Because different sources of fecal material can be associated with different types and levels of pathogens, the nature and loading of fecal source(s) are important variables to characterize. Further, characterizing whether the water body is predominantly contaminated with nonhuman fecal sources will be important to establish to support adjusting the 2012 RWQC. To begin, information on fecal sources and the hydrodynamics of fecal loading to the waterbody or waterbodies being characterized is first collected via a process termed "sanitary characterization" (Section 1.3.2). The sanitary characterization includes two steps: (1) a sanitary survey (Step 1) and (2) a water quality study (Step 2).

If the predominance of nonhuman fecal inputs is established, then the next step (Step 3) is to combine the information collected in Steps 1 and 2 with the information on pathogenicity and exposure, discussed in Section 2, by conducting a QMRA. The QMRA estimates the potential human health risks from primary contact recreation in the waterbody with predominantly nonhuman fecal inputs.

Step 4 describes how to derive alternative WQC using the information and results from Steps 1 through 3. The alternative criteria adjust the magnitude of the EPA's national recommendations to reflect the predominance of nonhuman fecal inputs. Any alternative WQC developed using this TSM will include a GM, STV, and a BAV consistent with the EPA's national recommendations.

Users of this TSM should understand the representativeness of the location chosen for conducting the sanitary characterization and the potential for applying the results of this TSM process beyond the location studied. Consider the variable fecal source loading dynamics, such as differences between baseflow and wet weather conditions or between tidal states. Site selection (including the consideration of unique watershed characteristics) for the sanitary characterization steps (including the data generated and, by extension, the QMRA results) can influence the decision on the applicability of the results beyond the study location. It is recommended that a sanitary characterization be conducted at multiple sites where the nonhuman fecal source is thought to predominate to document

the variability of conditions, fecal loading and resulting risk estimates. A multi-site characterization could provide additional substantiation to decisions on the broader applicability of results.

3.1 Step 1: Sanitary Survey: Identify the Contributing Fecal Sources

In Step 1, information about the fecal sources affecting the waterbody is collected through a sanitary survey and other available sources of information to help understand the potential sources affecting the waterbody more clearly (Text Box 3-1). The EPA developed a new QMRA sanitary survey form to guide data collection and documentation for this step. (Refer to Appendix B, QMRA Sanitary Survey Form.)

Text Box 3-1. Decision Point Step 1

Based on the available information, a conclusion is made that the predominant sources of fecal contamination impacting the waterbody are nonhuman. Include nonfecal source(s) of FIB if significant. Substantiate this conclusion using a sanitary survey and other lines of documented evidence.

The QMRA sanitary survey form can be augmented with other useful documentation (Figure 3-1). For example, the management history of the watershed might provide information on historical water quality and identify previous source control efforts. The history of BMPs and other mitigation efforts intended to attenuate fecal sources may also be useful information to document as part of the sanitary characterization. Impervious surfaces and constructed drainage features or other built structures are also useful information that may impact fecal sources.

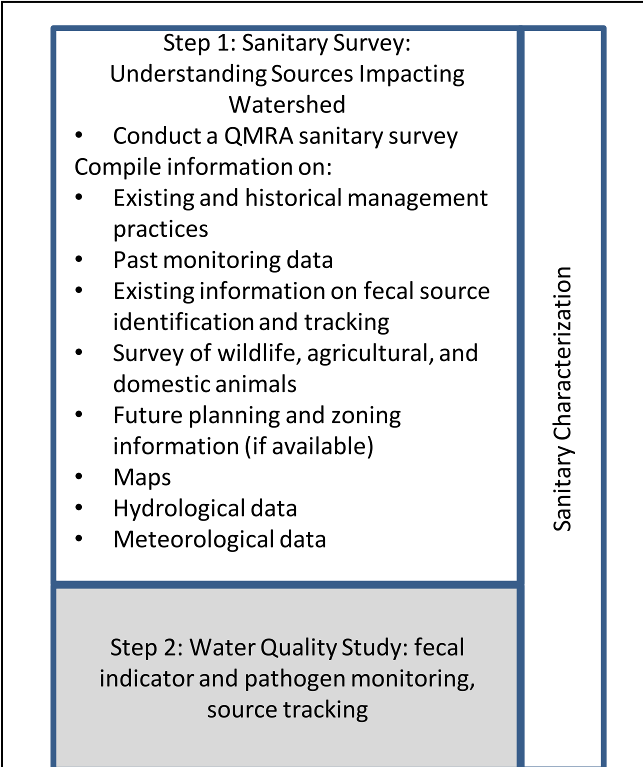


Figure 3-1. Information collected in Step 1.

(This figure shows details of Step 1 in Figure 2-6.)

The sanitary survey form in Appendix B includes three main sections:

1. A summary form at the top of the sanitary survey form to integrate information collected about potential fecal sources identified in the watershed that can affect the particular waterbody.
2. A watershed information form (Section 1) for documenting details about the watershed and existing water quality monitoring location(s).
3. A series of worksheets that are used to document details about potential sources of fecal contamination in a watershed or at a recreational site.
    a. Worksheet A: Description of land use, maps, and physical conditions.
    b. Worksheet B: Weather conditions, water quality sampling and modeling.
    c. Worksheet C: Human fecal sources.

d. Worksheet D: Stormwater, urban runoff, drains, streams, wetlands.

e. Worksheet E: Agricultural animals and wildlife.

An explanation for the data requested in specific sections is provided following the worksheets.

Any existing fecal source identification information can be helpful in identifying sources of FIB and pathogens (U.S. EPA, 2011a). Summarize the fecal source information, including the basis of the information, such as peer-reviewed publications, watershed reports prepared by local or state agencies, discharge permits, or waste load allocation information. The uncertainties, variability, and assumptions associated with the available fecal source identification studies need to be summarized clearly, concisely, and transparently. The relevance of the existing studies should also be described (e.g., how recent are the studies, are existing conditions similar to the conditions extant at the time of the study, did the existing study characterize the hazardous condition[s]) and focus on points that apply to the waterbody and are relevant with respect to microbial WQC. Existing data for microbial source markers can be useful for understanding the nature of fecal inputs in the watershed and can be included. Please note for the purposes of this TSM, source marker data do not necessarily reflect the potential risk from that source. Examples of RBTs for microbial source markers are available in the scientific literature (Brown et al., 2017a; Boehm et al., 2018; Wu et al., 2020; Boehm and Soller, 2020). The sanitary survey, coupled with the other information discussed in Figure 3-1, can be useful for establishing evidence of source(s) that can be confirmed in Step 2.

Document the occurrence and prevalence of wildlife (e.g., warm-blooded animals) that could be a source of FIB, pathogens, or both. For example, waterfowl can contribute substantially to fecal indicator loads (Hansen et al., 2011; Sinigalliano et al., 2013). Exposure to gull fecal contamination has been shown to pose significantly lower human health risks than exposure to human fecal sources at equivalent culturable FIB levels because the pathogen profile present in gull feces differs from the pathogen profile in human feces (Schoen and Ashbolt, 2010; Soller et al., 2010b). The differences in pathogen profiles relative to the loading of culturable FIB from each source result in a risk differential relevant to risk management decisions and public health protection. A wildlife survey can document the types of wildlife that are present, seasonal fluctuations, and other discernible wildlife patterns.

Sanitary surveys also include documentation of the occurrence of agricultural animals, such as livestock and poultry operations, recreational animals, such as horses, and the presence of domestic animals, such as dogs allowed at the beach. Published QMRA evaluating recreational exposure to the direct deposition of cattle feces into surface water demonstrates a similar magnitude of gastrointestinal illness risks as human fecal sources (Soller et al., 2010b); however, the risk from the indirect deposition, or rainfall-induced runoff, of cattle manure can be substantially lower due to differences in attenuation and mobilization of pathogens and FIB in the applied manures prior to rainfall events (U.S. EPA, 2010a; Soller et al., 2015). Effective manure management practices can encourage pathogen attenuation, reduce offsite transport of animal feces in runoff, and decrease fecal contamination of surface waters, so it may be important to record such practices if direct deposition might not represent the situation at the study location (ASCE and U.S. EPA, 2000; CWP, 2007).

Geographic information system (GIS) mapping can also provide important insights about a watershed. A map can be included in the initial sanitary survey identifying any WWTP outfalls or other features that could contribute (e.g., septic systems or pit toilets) or attenuate (BMPs) FIB and pathogens on the map. Note that a WWTP in the watershed is considered a significant human source even if treatment is

effectively reducing FIB. A properly designed and operated WWTP reduces culturable FIB more effectively than pathogenic viruses and protozoa; therefore, waters affected by effluent can pose elevated risks to human health that are not predicted by culturable FIB (Wade et al., 2006, 2008, 2010, 2022).

Any information on currents and tidal effects can help characterize how the FIB and pathogens move throughout the watershed. In some cases, hydrological data might be available in map format. This information can also be important for designing the sampling strategy for the Step 2 water quality study.

If the sanitary survey indicates that the sources appear to be predominantly nonhuman, and other factors such as public perception and available resources indicate that alternative criteria would be desirable and that downstream waters would still be protected, then proceed with Step 2, the water quality study. The information from Step 1 might also indicate the potential benefit of management actions (e.g., initiating manure management practices, installing fencing in the watershed to prevent livestock from having direct access to surface water, implementing sanitation practices at beaches to deter waste scavenging by birds and other animals) that could positively affect water quality. If this is the case, ideally, the management actions could be implemented before Step 2 is initiated. The EPA's Handbook for Developing Watershed Plans to Restore and Protect Our Waters walks users through the basic watershed planning and implementation process (U.S. EPA, 2008b, 2013c).

Alternatively, if the results of the sanitary survey indicate a higher contribution of human fecal contamination than initially thought, then a decision not to proceed with developing alternative criteria could be made. The presence of point sources, such as WWTP effluent discharges or combined sewer overflow (CSO) outfalls, can contribute significant loading of human enteric pathogens to surface waters. Higher densities of older septic systems in areas with poorly draining soils can also result in significant contributions of human fecal contamination (Peed et al., 2011). Published QMRA results have suggested human contributions of approximately 20%–30% as a potential threshold for determining if a water is predominantly affected by nonhuman fecal sources (Schoen et al., 2011; Soller et al., 2014). As the proportion of human fecal loadings increases, a clear understanding of how and when these inputs occur is needed so that the potential higher-risk scenarios these sources can cause are identified. Information on source loading dynamics can help inform the monitoring approach and analytical targets included in the water quality study discussed in Step 2.

3.2 Step 2: Source Confirmation: Conduct a Water Quality Study

In Step 2, a water quality study is conducted to characterize fecal indicators, source markers, and pathogens (Text Box 3-2). The water quality study can (1) be used to verify the results of the sanitary survey and (2) provide data for input into QMRA in Step 3. The reference pathogens chosen for monitoring during the water quality study can be based on the fecal sources identified in Step 1.

Ideally, the information collected during the water quality study supports the results of the sanitary survey—that the predominant fecal loading to the waterbody is nonhuman. The scope of the water quality study can include FIB and

Text Box 3-2. Decision Point Step 2

The collected information should characterize the source(s) of fecal contamination in the watershed. A conclusion is made that the predominant sources of fecal contamination affecting the waterbody are nonhuman. Substantiate this conclusion using the data collected via monitoring and modeling as part of the water quality study.

pathogen monitoring in the waterbody or waterbodies of interest, FIB and pathogen monitoring in the feces of the principal sources of concern, fate and transport modeling, and fecal source identification and tracking information, such as microbial source markers (Figure 3-2). The water quality study can also help investigators understand the magnitude of fecal loading and under what conditions the fecal loading occurs. The water quality study approach and design consider the fecal loading dynamics, and the monitoring program reflects the hazardous condition when the fecal loading is thought to occur. For example, if the fecal loading from a source is associated with wet weather, then the monitoring program would reflect wet weather events. For event-driven fecal loadings, monitoring the base flow conditions is not necessarily informative nor provides robust data for QMRA. Users are encouraged to consider monitoring at locations with the potential for fecal loading (e.g., if a nonhuman source is thought to enter surface waters via runoff, then sampling close to that location when runoff is occurring can be helpful to understand the potential fecal loading and contamination dynamics). Monitoring at locations proximal to the fecal loading can aid in enumerating pathogens that may occur in the source.

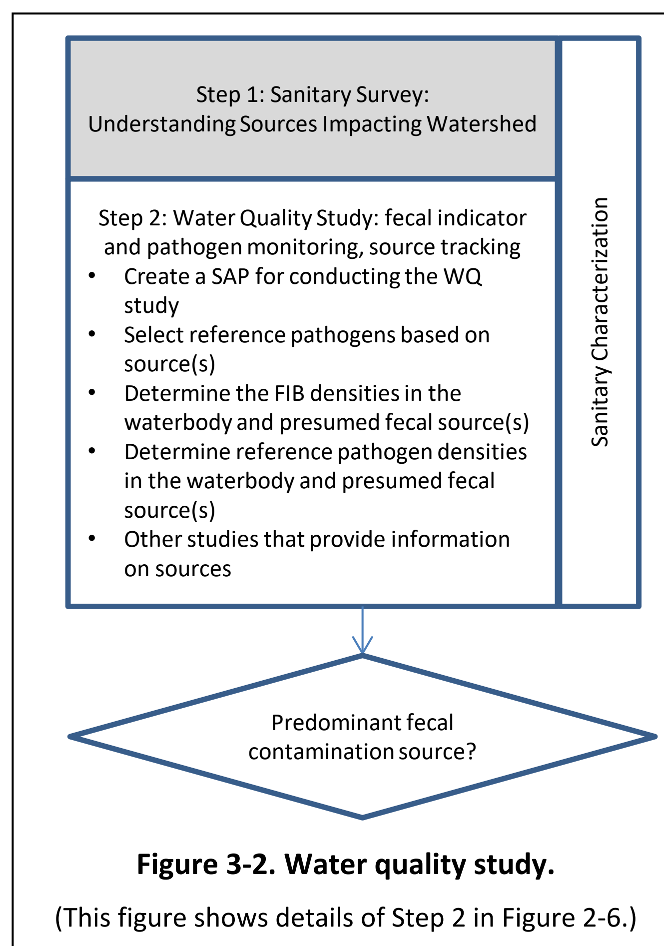


Figure 3-2. Water quality study.

(This figure shows details of Step 2 in Figure 2-6.)

To begin the water quality study process, users of this document develop a SAP, which describes the processes for characterizing water quality and fecal sources and analyzing the data collected, including quality assurance plans. The SAP should be designed to capture the hazardous conditions where the identified fecal sources in Step 1 are thought to contribute to the loading of fecal material to surface waters. If fate and transport parameters, such as accounting for mobilization or environmental decay, or integrated environmental modeling (IEM) are anticipated, the SAP discusses the data being collected and how it is used in a model. Appendix C includes an example SAP used to characterize levels of FIB, source markers, and pathogens in a study the EPA conducted to support a QMRA at a beach in Boquerón, Puerto Rico. Having a detailed SAP and having subject matter experts review it can help ensure the scientific defensibility of the effort. Quality assurance procedures for data collection are also an important part of the SAP. The SAP is expected to be representative of the meteorological and hydrological conditions in the watershed. The EPA has published guidance on systematic planning using the data quality objectives (DQO) process (U.S. EPA, 2006b). The DQO process can be helpful during planning when environmental data are used to estimate contamination levels and inform decision-making. Refer to Chapter 7 of U.S. EPA (2006b), “Step 7: Develop the Plan for Obtaining Data,” to inform the development of a SAP for the water quality study.

Fecal source identification information is important evidence for demonstrating that predominant impacts on the site are nonhuman. Document the specificity and selectivity of source markers used for

fecal identification. It is recommended to evaluate fecal samples or discharges from the presumed source(s) to test that the source identification markers chosen can reliably detect that source. For example, if birds are suspected of being an important source, test the marker on fecal samples from the birds thought to be a contributing source. This is an active area of research, and examples of relevant studies include Boehm et al. (2013), Shanks et al. (2010, 2016), Sinigalliano et al. (2013), Unno et al. (2018), and Holcomb and Stewart (2020). The fecal source identification characterization can indicate whether the sources are predominantly nonhuman and, in turn, inform the selection of reference pathogens for that waterbody.

When the information collected in Step 1 indicates that humans are not a contributing fecal source, the EPA recommends using human MST marker(s) to confirm that significant loading of human fecal material is not occurring. A pattern of positive results in the water quality study using a human marker when the sanitary survey indicated little to no human inputs suggests that potential human source(s) contributing to the waterbody may have been missed; thus, it might be necessary to revisit the sanitary survey step or reevaluate the study site as a location to collect data supporting the development of alternative criteria. If source markers indicate human inputs (qualitatively or quantitatively), it is important to have a better understanding of the potential magnitude and contamination pattern of the human input. The presence of human markers below the LOQ of the detection methods is best evaluated in context to the overall fecal loading to a waterbody. Detection of microbial source markers does not necessarily indicate elevated human health risks, but marker detection can, for example, inform on the monitoring approach in the water quality study and choice of reference pathogens to include in the QMRA.

Text Box 3-3. Reference Pathogens

Reference pathogens represent the pathogens that are most likely to occur in waterbodies with warm-blooded animal (including human) fecal sources. *Campylobacter* spp., *Cryptosporidium* spp., *E. coli* O157:H7, *Giardia* spp., and *Salmonella enterica* represent pathogens that are potentially zoonotic and associated with human and nonhuman fecal contamination (U.S. EPA, 2009a). Adenovirus, norovirus, and rotavirus are associated with human fecal material and are not zoonotic. This set of reference pathogens is useful because it covers the major waterborne pathogens associated with non-foodborne illness occurrence in the United States.

Select reference pathogens that reflect the enteric pathogens potentially present in the identified fecal sources. The EPA is providing information on the standard QMRA reference pathogens adenovirus, *Campylobacter* spp., *Cryptosporidium* spp., *E. coli* O157:H7, *Giardia* spp., norovirus, rotavirus, and *Salmonella enterica* (Text Box 3-3). For example, if gulls are the source of the fecal contamination, the pathogens of concern are *Salmonella enterica* and *Campylobacter* spp. For pigs, the pathogens of concern are *E. coli* O157:H7, *Campylobacter* spp., *Salmonella enterica*, *Cryptosporidium* spp., and *Giardia* spp. However, users of this TSM are not limited to these reference pathogens. The EPA selected these reference pathogens to represent waterborne illness in recreational waters for several reasons: (1) they represent the three classes of pathogens of concern (viruses, protozoa, and bacteria); (2) they are found in a wide variety of sources; (3) they have associated peer-reviewed dose-response relationships in the literature; and, (4) they account for more than 97% of nonfoodborne illness in the United States (Mead et al., 1999; Scallan et al., 2011a,b). Thus, these pathogens can be considered responsible for the largest proportion of nonfoodborne illness as reference pathogens for waterborne illnesses. The reference pathogens norovirus, adenovirus, and *E. coli* O157:H7 are also considered index pathogens because they represent a broader class of pathogens that share similar infectivity or environmental

fate and transport characteristics (WHO, 2004, 2005; Soller et al., 2010a; Ferguson et al., 2008). Using an index pathogen to represent a broader group of pathogens is a widely accepted practice in the field of QMRA (Roser et al., 2007; Soller et al., 2010b; Schoen et al., 2011).[18] Research has demonstrated that the bacteria (*Campylobacter* spp., *E. coli* O157:H7, and *Salmonella enterica*) and protozoa (*Cryptosporidium* and *Giardia* spp.) on the list can be zoonotic pathogens, that is, pathogens transmitted from animals to humans. For more discussion of these pathogens, see Section 2.1.1.

In cases where the fecal source information collected demonstrates little to no human fecal inputs, a decision not to include the virus reference pathogens (specifically adenovirus, norovirus, rotavirus) could be made because these are exclusively associated with human sources. However, in this case, limited monitoring for the reference viruses could be conducted to evaluate the assumption that there are little to no human fecal sources. It is recommended that human MST markers, such as HF 183 (U.S. EPA, 2019b), be used for this evaluation because MST markers tend to occur in higher abundance and with more consistency compared to enteric viruses. When monitoring for both enteric viruses and human markers, human markers may be detected and quantified, but viral pathogen monitoring may have many nondetects due to the abundance difference and because the pathogens might not be circulating in the population contributing to the source (e.g., seasonal occurrence of norovirus in sewage). For suspected human source contributions, investigators might want to consider increasing sample volumes in combination with ultrafiltration (Bofill-Mas and Rusinol, 2020; Korajkic et al., 2022). Designing a monitoring approach to reflect when a suspected human source is reaching a surface water can help characterize the magnitude of human fecal loading; see Zimmer-Faust et al. (2020) for an example of a high-resolution sampling approach. Collecting samples at the point of discharge or proximal to where the suspected source reaches surface water can aid in the detection of pathogens. The other five reference pathogens (i.e., bacteria and protozoa) can be of zoonotic origin (U.S. EPA, 2009b). Using this information, select the reference pathogens based on the fecal sources identified and/or suspected.

The water quality measurements for FIB, MST markers, and pathogens are considered because these data are needed to characterize current conditions at the study site(s). Describe which FIB, MST, and pathogen data are being collected in the SAP. The environmental monitoring data collected from the study site(s) will be used in the next step (Step 3). In waters contaminated by feces, pathogens can be present in much lower densities than FIB and MST markers. Pathogen occurrence in feces can be transitory and affected by population prevalence and lifestage, which is partly why surrogates of fecal contamination like FIB are relied on to routinely serve as metrics for water quality determinations rather than pathogens (Text Box 3-3). Microbial enumeration methods tend not to be efficient when low levels of the target are present and can have poor recovery. Characterize and describe the recovery efficiency enumeration methods as part of the SAP development. Discuss enumeration method LOD and LOQ in the SAP.

Process monitoring data for FIB and pathogens by handling nondetects and samples below the LOQ in a defensible and transparent manner. LOD is the level below which the method does not detect the presence of microbes. A “nondetect” result does not necessarily mean that the actual sample value is zero but rather that the microbial target is present below the LOD of the assay used (Silvestri et al.,

[18] Definitions are:
Reference pathogen: A pathogen responsible for a significant proportion of waterborne gastrointestinal illness.
Index pathogen: A pathogen that does not necessarily cause a large proportion of illnesses but represents a broader group of pathogens that as a group are important.

2017). The LOQ is the level at which the method can enumerate the microbe. For culture-based methods, the LOQ is usually reported as a countable range, such as 20 CFU–200 CFU for membrane filter plates (Silvestri et al., 2017). Below the LOQ, the method may detect but cannot accurately enumerate the organisms. Above the upper level of the LOQ, the method cannot enumerate accurately due to factors like overgrowth or colony growth interference (Be'er et al., 2009; ASTM, 2020). Problems with upper LOQ can be attenuated by diluting the sample, so only the lower LOQ is considered in the discussion that follows. For assays that enumerate microbes using culture methods, the LOD can be the LOQ. Molecular methods, such as qPCR, can have an LOD that is below the LOQ. Therefore, discussing both the LOD and LOQ of qPCR assays is essential. Understanding these thresholds is important for preparing datasets for analysis.

Some water quality monitoring results may be below the LOQ or below the LOD. Depending on whether enumeration of FIB or pathogens is used, results that are less than the LOD or LOQ will be treated differently. FIB are often present at densities at or above commonly used assay LODs (which in most cases is also the LOQ). For this reason, many samples will have an actual measured density of FIB. Also, higher variability is expected below the lower end of the LOQ due to various biological, method and matrix factors (Silvestri et al., 2017). Occasionally, a sample might be below the LOD. The scientific literature contains precedent for replacing results below the LOD with the LOD, or ½ the LOD, although more complex replacement methods exist. Replacing nondetect values for FIB with the LOD or ½ LOD can be used, depending on the goal of subsequent statistical analysis. Provided the substitution does not drastically change the data distribution including central tendency measures or the standard deviation (SD), substitution will likely not significantly affect the analyses. For FIB, the nondetects are most likely in the lower tail of the water quality distribution. Provided the replaced LOD values represent a small fraction of the total data observations (< 15%) (U.S. EPA, 2000b), as is likely for FIB data used in this context, replacement is acceptable. See Site-Specific Alternative Recreational Criteria Technical Support Materials for Alternative Indicators and Methods for additional discussion of handling nondetects for FIB (U.S. EPA, 2014a).

The ability to detect and quantify pathogens in surface waters is influenced by their occurrence and prevalence in the population that is contributing to the fecal loading to a waterbody. If there are few infected individuals excreting the pathogen, the population of infected excreters is small, or amount of feces excreted per infected animal is low, then the overall pathogen loading to a waterbody can be low. Pathogen occurrence may be affected by lifestage (e.g., super-shedding of *Cryptosporidium* and *E. coli* O157:H7 by cattle) or season (Lal et al., 2012; Thomson et al., 2019; Antaki-Zukoski et al., 2021). Because of the complex environmental/host/pathogen interactions, pathogens might be present in ambient waters at densities near or below the assay LOD. For example, it is common in environmental datasets for pathogens to be "ND" in more than 50% of samples assayed. In such cases, replacing values below the LOD with the LOD or ½ LOD (or LOQ) is not advised because it would drastically change the data distribution. The EPA addressed this issue when estimating densities of *Cryptosporidium* in U.S. drinking water sources (U.S. EPA, 2005). The goal was to obtain the distribution of *Cryptosporidium* densities in water using densities measured across the country. Many of the measurements, however, were below the LOD. The EPA used a Monte Carlo simulation to estimate densities given the volumes of samples the participants assayed, the participating lab's recovery, and the count of *Cryptosporidium* oocysts obtained for the various samples (many were zero) (U.S. EPA, 2005). This technique resulted in a data distribution despite the numerous measurements that were below the LOD. Alternatively, if the fecal sources affecting the waterbody were shown to be predominantly nonhuman and the human fecal inputs do not exist or are negligible, nondetects can be

replaced with zero for the human viral pathogens. If results are above the LOD but below the LOQ, replacing those results with the LOQ is likely acceptable. With molecular analytical methods in particular (for both pathogens and MST markers), a transparent description and documentation of the LOD and LOQ thresholds is critical for demonstrating that data were prepared for analysis in a scientifically defensible manner. For some pathogen enumeration methods with high LOQs, generating quantitative environmental data may be challenging without applying analysis methodologies to support data interpretation. This example illustrates the importance of the sanitary characterization for this approach. Refer to Helsel (2012) for additional approaches for including nondetects in environmental datasets.

Step 3 involves fitting the microbial data to a statistical distribution for input into the QMRA. A common distribution for this purpose is a lognormal distribution (Text Box 3-4), which can be fully characterized in terms of an estimated GM and the logSD.[19] For example, the EPA recommended water quality values are expressed as a GM of a lognormal distribution. The GM and logSD of the distribution can be readily computed from the data collected in Step 2. A spreadsheet such as one in Microsoft (MS) Excel[20] is an effective and straightforward tool for this purpose. For example, using MS Excel, compute the base10 log of each enterococci observation (the syntax in MS Excel is =log10(observation)). Then calculate the average (=average(log10(observations))), and the SD of those log values or the logSD (=stdev(log10(observations))). The GM of the distribution is obtained by computing the antilog of the computed average of the log values, and the geometric SD of the distribution is obtained by computing the antilog of the computed logSD.[21]

Text Box 3-4. Lognormal Distribution and Geometric Mean

Water quality at a beach tends to be lognormally distributed because water quality data typically span many orders of magnitude, and the distributions are right-skewed since they are bound by zero on the left (Bartram and Rees, 2000; Kay et al., 2004; Wyer et al., 1999). General environmental sampling data tend to follow lognormal distributions, a phenomenon discussed by Esmen and Hammad (1977). The data sets from some beaches may not fit a lognormal distribution. However, based on data from the NEEAR beaches and other studies from the literature, when enough data are collected and the samples below the detection limit are treated in a statistically rigorous manner, the lognormal distribution tends to be a good fit for water quality monitoring. For example, WHO based their recreational water Guidelines (WHO, 2003) on a lognormally distributed dataset from the EU from over 11,000 bathing locations and over 121,000 enterococci observations (Kay, et al., 2004).

The use of the GM for RWQC is discussed in detail by Wymer and Wade (2007). Numerous researchers have used the GM to discuss the central tendency of water quality at beaches (for examples, see Boehm et al., 2009 and Whitman et al., 2004). EPA's 2012 RWQC recommends GM water quality values because the GM is significantly associated with gastrointestinal illness in epidemiological studies. Note that this health relationship is not tied to individual sample results.

[19] The logSD is the SD of the base10 log of the data. The antilog of the logSD would be the geometric SD.
[20] Microsoft. *Microsoft Excel*. Redmond, Washington: Microsoft, 2010. Computer Software.
[21] This information will be used in Section 3, Step 3 (below) to calculate the density of FIB in the waterbody.

The data and information from Steps 1 and 2 together constitute a sanitary characterization and provide evidence about the sources of FIB and pathogens in the watershed sufficient to decide that the predominant source affecting the waterbody is nonhuman. Waters with a greater susceptibility to human fecal contamination would need a greater weight of evidence to demonstrate that nonhuman sources are predominant. Depending on the level of evidence needed, approaches for demonstrating source loading can range from qualitative to quantitative. Apportioning sources quantitatively solely based on source marker data is not a straightforward process, although research in this area is ongoing. However, multiple approaches exist, including source marker, FIB, watershed, and meteorological information providing evidence of fecal contamination dynamics. Investigators have used source marker data combined with GIS land use and *E. coli* measurements to characterize fecal contamination trends on a watershed scale (Li et al., 2019). Human, canine, and avian source marker data were evaluated in combination with FIB and precipitation information to identify sources associated with an exceedance of the BAV (Shrestha et al., 2020). In another study, paired measurements of viral and bacterial fecal indicators and human, ruminant, canine, and avian source markers were assessed from six Great Lakes locations over a recreation season to estimate fecal score ratios (Li et al., 2021). Approaches for estimating fecal mass loading from a watershed to a waterbody and the process for developing TMDL apportionment can also be applied to inform this decision (U.S. EPA, 2001).[22]

The EPA's researchers have employed recursive partitioning (RP) to evaluate MST marker and FIB data (Bradshaw et al., 2016; McKee et al., 2020, 2021) using water quality data that are log-transformed prior to linear regression. In Bradshaw et al. (2016), RP was used to predict pathogen presence based on an analysis of independent variables. RP analysis assigned a score to the identified independent variables to define the level of association. Inconsistent relationships between individual pathogens, FIB and source markers were found. However, in a mixed land use system, a combination of FIB, MST, and other water quality measurements, such as pH and water temperature, were helpful in assessing microbial water quality (Bradshaw et al., 2016). RP can also be used to determine which marker, and at what level, best predicts the presence of pathogens or density of FIB. For example, McKee et al. (2020) found the dog source marker (DogBact) had the largest R^2 value and was the best predictor of elevated *E. coli* levels above the BAV. McKee et al. (2021) evaluated the spatial and temporal patterns of fecal contamination in Congaree National Park, SC, which is affected by feral swine populations. The swine marker was the most frequently detected of the various source markers included. Swine marker levels above 43 gene copies per mL were associated with *E. coli* levels above the BAV (McKee et al., 2021).

The EPA is not suggesting a specific maximum allowable quantitative level of human fecal contamination for developing alternative values for culturable *E. coli* or enterococci because there is a wide range of variability of potential risk among human fecal sources (e.g., secondary treated and disinfected effluent, raw or poorly treated sewage, septage), so the level of human fecal inputs that would qualify as human predominated is dependent on the nature and magnitude of the human inputs. However, the level of human fecal contamination is generally addressed in this TSM by evaluating the susceptibility of the waterbody to human inputs. As discussed in Steps 3 and 4, evaluation of human and nonhuman fecal mixtures and recreational exposures demonstrated a lower risk of gastrointestinal illness when human fecal contributions were less than approximately 30% of the mixture (Soller et al., 2014). For the purposes of this document, susceptibility to human fecal contamination is considered "low" when the human contribution is below approximately one-third of

[22] For the EPA's guidance on TMDLs, see https://www.epa.gov/tmdl (last accessed May 25, 2023).

the total fecal loading to a waterbody. As the proportion of human contribution increases, human enteric viruses begin to dominate the overall estimated risk (Schoen et al., 2011; Soller et al., 2014). Two additional categories of human fecal susceptibility, "medium" (34%–67% human contribution) and "high" (> 67% human contribution), have results similar to the 2012 RWQC recommendations (see examples in Figure 3-8 and Table 3-2) (U.S. EPA, 2010a; Soller et al., 2014). The EPA recommends that only waters falling into the low-susceptibility category be considered for alternative WQC. The uncertainty associated with source apportionment and risk estimates will be affected by the quantity and quality of the data collected. The range of variability associated with the data collected and the implications of uncertainty for the decision-making process should be evaluated and understood. As the human fecal contribution increases, the calculated indicator value will approach that of the 2012 RWQC, which is based on data collected in waters predominantly affected by human fecal sources. Temporal variability of source contributions can be considered as part of source determination. Two examples of how the sources of fecal contamination might vary over a year include manure slurry applications during winter or sewer overflows during seasonal wet weather. The EPA and others have demonstrated that nonhuman fecal sources can pose less potential for human health effects at the same level of fecal indicator (Schoen and Ashbolt, 2010; Soller et al., 2010b; U.S. EPA, 2010a; WERF, 2011). Consider the information from Steps 1 and 2, along with stakeholders' feedback, when deciding whether to proceed with the alternative criteria development process.

3.3 Step 3: HHRA—Forward and Relative QMRA modeling.

Step 3 uses the information collected in Steps 1 and 2 to estimate the potential human health risks associated with recreational exposure to the waterbody. In this step, the risk assessment process is considered a "forward" QMRA because pathogen occurrence is an input for calculating a health risk estimate as the output. This step describes two forward QMRAs (Figure 3-3). The outputs from both approaches are then compared to the target illness rate chosen and the EPA's recommended water quality values to inform a decision on proceeding with developing alternative criteria values (Text Box 3-5).

Text Box 3-5. Decision Points Step 3

- What target illness rate and water quality values are chosen?
- How do the QMRA estimates of illness compare to the target illness rate?
- How does the water quality at the study location compare to the recommended water quality values?
- Do the QMRA estimates support proceeding with developing adjusted water quality criteria?

Conduct the Step 3 human health risk evaluation using the QMRA model parameters summarized in Section 2.1 and the data collected in Section 3.2 (Step 2) in two different but complementary approaches to estimate the waterbody pathogen density:

1. Estimate the waterbody pathogen density for each reference pathogen based on the reference pathogen data gathered in Step 2.

2. Estimate the waterbody pathogen density for each reference pathogen from the FIB density in the waterbody, FIB density in the source, and reference pathogen density in the source using the methods of Schoen and Ashbolt (2010) and Soller et al. (2010b).

As mentioned in Step 2 (above), pathogen levels in receiving waters can vary over time and space, and the recovery efficiency of pathogen enumeration methods can be poor, particularly when levels of pathogens are low in ambient waters. Therefore, estimate the densities of reference pathogens based

on both data collected and the range that the literature reports in fecal material (Section 2.1.1.1). Both approaches are reasonable ways to address the evaluation of potential human health risks. Literature values can be used to provide an upper-bound estimate for the potential illness. In both cases, the pathogen densities and the estimates of the volume of water ingested during recreation can be gathered from a dose-response relationship from the peer-reviewed literature and the conditional probability of illness given infection from the literature to estimate illness levels. Results from these two approaches might indicate a range of potential human health risks, or they could yield comparable results (e.g., pathogen data collected in the waterbody or the identified fecal source(s) might be in the same range as reported in the peer-reviewed literature). More detail on each approach can be found in Sections 3.3.1 (Approach 1) and 3.3.2 (Approach 2). Guidance for interpreting the results of both approaches can be found in Section 3.3.3.

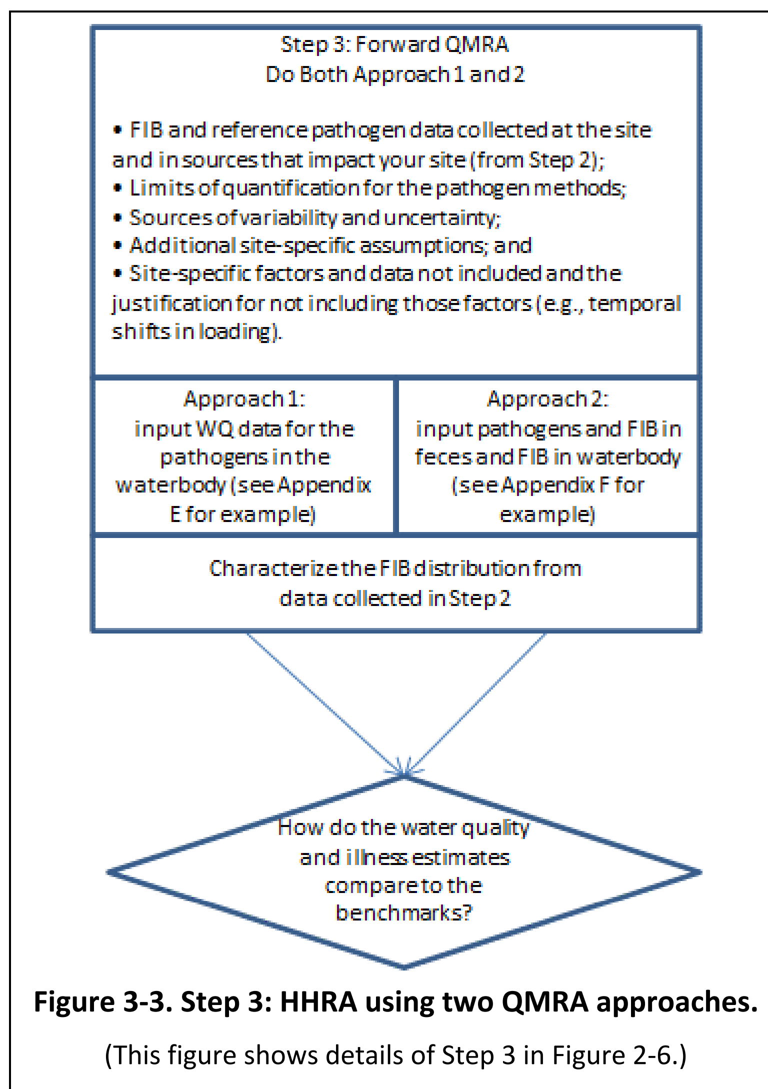


Figure 3-3. Step 3: HHRA using two QMRA approaches.

(This figure shows details of Step 3 in Figure 2-6.)

As discussed in Section 2.2.2.4.1, the EPA developed an illustrative exposure scenario for use in the QMRA analyses recommended in this TSM. The illustrative scenario assumes the direct deposition of feces to a waterbody in close proximity to recreation. Users of this TSM may be interested in incorporating fate and transport terms to describe the behavior of the pathogens, FIB, and microbial source markers from the source to the study location. Refinement of the illustrative scenario with additional fate and transport information, such as including indirect fecal deposition pathways, is possible, but be aware that specific fate and transport characteristics in the watershed studied may limit the scope of the applicability of the QMRA output. Using fate and transport modeling or mobilization data to estimate pathogen densities in the studied waterbody can introduce additional complexity and a wider range of assumptions compared to the direct deposition scenario described by the EPA. A more generic indirect fecal deposition scenario was developed by the EPA in which animal manures were land applied at agronomic rates and mobilized by rainfall (U.S. EPA, 2010a; Soller et al., 2015). In this scenario, mobilized fecal pathogens were characterized at the edge of the area where manures were applied. Pathogen densities in the runoff were used to estimate risk. This indirect deposition scenario incorporates a common mechanism—wet weather-induced mobilization of manures—and could be utilized if, for example, grazing animals are an identified fecal source in the watershed studied and do not have direct access to the waterbody studied or its tributaries. The EPA

decided to use a simplified exposure scenario (i.e., direct deposition) in its approach, given the variability and uncertainty associated with the input parameters.[23]

The illustrative exposure scenario may not be representative for the user's study location, and there may be interest in incorporating fate and transport because primary contact recreation occurs downstream a significant distance from the predominant contamination source. To consider the various decision points, the EPA has developed an IEM framework (Figure 3-4) that includes fate and transport considerations and the health models used in QMRA to link the risk estimation between source and receptor locations in a watershed (Whelan et al., 2014a,b; U.S. EPA, 2017a,b,c,d,e,f,g,h,i). The IEM approach is more data-intensive than the approach described in this TSM, but both can provide quantitative risk estimates that can inform decisions on proceeding to Step 4.

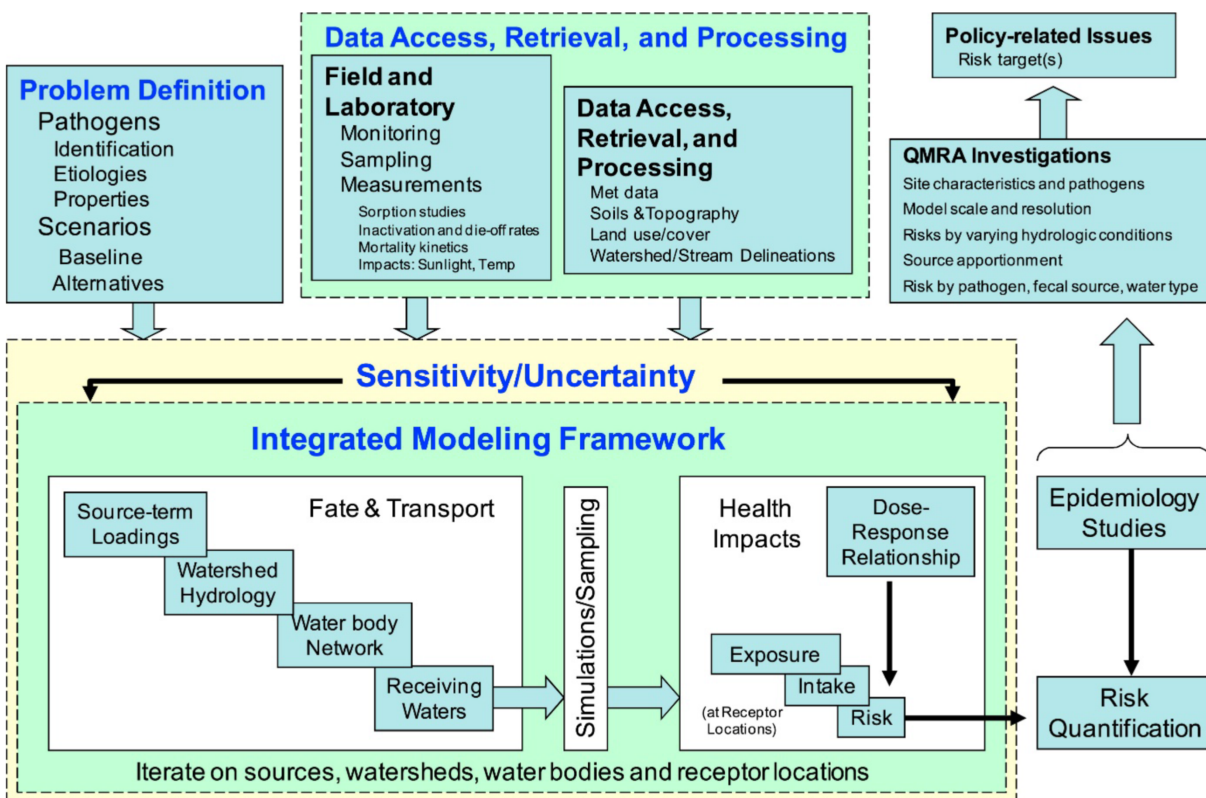


Figure 3-4. An example of an integrated, multimedia modeling framework linking the problem definition, data access, retrieval, and processing, and IEM (including the health models used in QMRA) to quantify risk at receptor locations (adapted from Whelan et al. [2014a]).

[23] Variability is differences attributable to true heterogeneity or diversity in a population or exposure parameter. Variability implies real differences among members of that population. For example, different individuals have different intakes and susceptibility. Intraindividual variability refers to differences over time for a given individual. Interindividual variability refers to differences across members of a population at a given time. Variability in MRA cannot be reduced, but it can be more precisely characterized. Uncertainty occurs because of lack of knowledge regarding the true value of a quantity, such as a specific characteristic (e.g., mean, variance) of a distribution for variability, or regarding the appropriate and adequate inference options to use to structure a model or scenario. The EPA also refers to these as model uncertainty and scenario uncertainty. Lack of knowledge uncertainty can be reduced by obtaining more information through research and data collection, such as through research on mechanisms, larger sample sizes, or more representative samples (U.S. EPA, 2007).

Rather than recommending one specific method for modeling this type of exposure scenario, the EPA suggests using health-protective assumptions to reduce complexity. If fate and transport parameters are included, it is important to thoroughly characterize and carefully document the inclusion of fate and transport information at all steps (FIB and pathogens on a site-specific basis). Also, when including specific fate and transport information, it would be important to consider the applicability of the resulting illness estimates more broadly if the information is used to develop alternative criteria discussed in Section 3.4 (Step 4).

3.3.1 Forward QMRA Approach 1: Using Data Collected from Water Quality Monitoring

In Approach 1, the reference pathogen data collected in Step 2 water quality study is used to estimate the waterbody pathogen density, which is then used in a forward QMRA to estimate human health risk from recreational exposure. A QMRA example (U.S. EPA 2010a; Soller et al., 2015) is described to illustrate the process associated with Approach 1. Although the analysis presented in the example was not conducted for a specific waterbody, it does follow the steps in the forward QMRA Approach 1 (see Section 2.2.2.4.3). The following data are needed for the forward QMRA process in Approach 1:

1. Reference pathogen densities: these data were collected as part of the water quality study in Step 2.
2. Volume of water ingested: data for incidental ingestion of water while recreating is provided in Section 2.2.2.2: Measures of Exposure.
3. Reference pathogen dose-response parameters: this information can be found in Section 2.1.1.3.2: Zoonotic Infectivity Potential.
4. Probability of illness given infection for each reference pathogen: this information can be found in Section 2.1.1.3.3: Morbidity.

A forward QMRA consists of three steps: (1) a calculation of a dose for each reference pathogen; (2) a calculation of the probability of infection for each reference pathogen; and (3) a calculation of the probability of illness given infection for each reference pathogen. The probability of illness for each reference pathogen is then summed to calculate a total estimated risk of illness from recreational exposure.

U.S. EPA (2010a) and Soller et al. (2015), hereafter referred to as "Example 1," discuss a forward QMRA conducted to estimate risk from recreational exposure to a hypothetical waterbody receiving rainfall-induced runoff containing cattle, pig or chicken fecal material that was land applied at recommended agronomic rates. In the scenario characterized in Example 1, FIB and reference pathogens were mobilized by the rainfall and moved offsite to an adjacent waterbody where primary contact recreation exposure occurs via incidental ingestion of undiluted runoff. This scenario was intentionally constructed to provide health-protective estimates of risk (U.S. EPA, 2010a). The reference pathogens included in the analysis were *Cryptosporidium* spp., *Giardia lamblia*, *Campylobacter jejuni*, *Salmonella enterica*, and *E. coli* O157:H7. Human enteric virus reference pathogens, such as norovirus or adenovirus, were not included because only nonhuman fecal sources were being characterized.

In Example 1, note that the actual parameter values used were current at the time of publication, and there are differences in some of the values discussed in Soller et al. (2015) compared to the values presented in this TSM (Section 2.1). For some parameters, newer information was available in the

scientific literature and has been included and discussed in this TSM. It is recommended that the user of this TSM be aware of how these values are incorporated into a forward QMRA (Figure 2-8) and be familiar with the equations in Section 2.2.2.4.3. Over time, new information for these parameters may be published in the peer-reviewed literature, and users could evaluate the quality of that information and include it in the QMRA analysis when this occurs as needed. It is worth noting that the newer published information included in this TSM did not result in substantial changes in the risk output or the characterization of a QMRA analysis for recreational exposure to gull feces (see Section 4.2 for a comparison).

In both Example 1 and the forward QMRA R code supplied in Appendix E, equations for each step of a forward QMRA are combined so that reference pathogen densities, either user-supplied from data collected in Step 2 or estimated using pathogen mobilization rates as in the example, are combined with the literature-based parameter values to provide a total probability of illness as an output. The R code uses a stochastic approach (some inputs are distributions), but users could also use a deterministic approach with point estimates as inputs using the equations in Section 2.2.2.4.3. For transparency, the three steps are described in more detail below, and the example is referenced to illustrate the process.

Calculation of dose of pathogen ingested. The dose of each reference pathogen will be influenced by the density of the pathogen in the water. Water quality data collected in Step 2 are used to determine the summary statistics (i.e., mean density, median density, minimum and maximum density, and the SD) for each reference pathogen (Text Box 3-3). In equation 1A (Section 2.2.2.4.3) and in the forward QMRA R code provided in Appendix E, the pathogen density is multiplied by the volume of water incidentally ingested (Section 2.2.2.2) to estimate a dose. Equation 1A shows both pathogen density and ingested volume as a point estimate rather than a distribution; however, whether a point estimate or a distribution is used, the basic relationship the equation describes is the same. The forward QMRA R code (Appendix E) combines user-supplied pathogen data with the exposure distribution parameters for the volume of water ingested to calculate a dose. To use this R code, the user supplies (1) the log mean and logSD of a distribution of incidental ingestion volume and (2) a spreadsheet containing monitoring data for pathogen density collected in Step 2. Note the R code requires that the pathogen data be in units per L, rather than units per 100 mL. The R code implements a Monte Carlo approach, which runs 1,000 iterations of the calculations. In each iteration, random values for each of the model parameters are drawn and used in the mathematical formulas.

In the example, direct pathogen measurement in surface water receiving runoff containing animal manures was not conducted. Manures can have low and variable levels of pathogens (Soller et al., 2015). The EPA conducted field experiments to empirically determine mobilization rates of reference pathogens and FIB from land-applied manures. The mobilization rates were combined with literature-based values for the proportion of animals shedding each pathogen and the density of the reference pathogens in cattle, pig and chicken manures to provide an estimate of pathogen densities in water (Soller et al., 2015). The approach used in the example included the following assumptions: intense rain-inducing runoff, no attenuation of the pathogen between runoff and entry into surface water, and ingestion of undiluted runoff (Soller et al., 2015). Also, in the example, the volume of water ingested was modeled as a log-normal distribution with a log mean and logSD of 2.92 mL and 1.43 mL, respectively (equivalent to a GM of 18.5 mL per recreational event; Dufour et al., 2006). Although the approach described for characterizing pathogen levels is more complex than what the TSM discusses in Step 2, the additional complexity helped address the variable nature of pathogen occurrence in animal

fecal materials and resulted in scientifically defensible estimates of pathogen densities in runoff from the experimental plots. The pathogen density estimates were then used to calculate a pathogen dose given incidental ingestion, as discussed above.

Calculation of probability of infection from an exposure event. The dose of each reference pathogen is then used to estimate the probability of infection from each reference pathogen. Equation 1B (in Section 2.2.2.4.3) shows that the probability of infection is a function of the dose-response relationship. The dose-response information for each reference pathogen can be found in Table 2-5 in Section 2.1.1.3.2. The forward QMRA R code provided in Appendix E includes the dose-response models (e.g., Beta Poisson, Exponential, and Hypergeometric) and the pathogen-specific parameters that are used in the model (e.g., alpha, beta, rho, eta are unitless parameters). The Approach 1 R code includes dose-response information for *Salmonella*, *Cryptosporidium*, *Giardia*, *Campylobacter*, adenovirus, and norovirus.

In the forward QMRA Example 1, the dose for each reference pathogen calculated above was combined with the dose-response function to calculate the probability of infection for each reference pathogen. It was assumed that infection from each pathogen is independent of the others (Soller et al., 2015).

Calculation of probability of illness given infection and total gastrointestinal illness rate. The probability of infection from each reference pathogen is multiplied by a pathogen-specific morbidity ratio to produce a probability of illness (U.S. EPA, 2010a). The morbidity factor is the percentage of infections that lead to illness for each reference pathogen. Equation 1C (in Section 2.2.2.4.3) shows how the probability of infection multiplied by the proportion of infected individuals that become ill results in the probability of illness. Table 2-6 in Section 2.1.1.3.3 includes literature-reported values for the proportion of infections resulting in illness for each reference pathogen. Appendix E's forward QMRA R code includes point estimates for the probability of illness, given infection for each reference pathogen. The last step in the R code sums the risks from all the reference pathogens and calculates the mean and median illnesses per 1,000 events, which is the output of the forward QMRA model. This summing step is shown in Equation 1D in Section 2.2.2.4.3. The median illness rate is used to compare to the EPA's recommendations in Section 3.3.

In the example, the total probability of illness was calculated for three separate hypothetical recreational exposure scenarios for waters containing cattle, pig, or chicken fecal material. The total risk is displayed as a total gastrointestinal illness rate, and the GM culturable enterococci density is shown in Figure 3-5 (Soller et al., 2015). The predicted median risk of illness from a recreational exposure to the undiluted runoff from each of the land-applied fecal materials was: (1) 46 illnesses per 1,000 recreation events with an associated GM 14,000 enterococci CFU per 100 mL from runoff containing cattle manure; (2) 15 illnesses per 1,000 recreation events with an associated GM of 36,000 enterococci CFU per 100 mL from runoff containing pig feces; and (3) 18 illnesses per 1,000 recreation events with an associated GM of 1,800 enterococci CFU per 100 mL from runoff containing chicken feces (Soller et al., 2015). In Figure 3-5, the line in the middle of the box is the median. The upper and lower edges of the box are the 75th percentile and the 25th percentile, respectively. The bars (whiskers) extend to the 90th and 10th percentiles. The diamonds are at the 95th and 5th percentiles. The dotted line represents the EPA's recommended target illness rate of 36 NGI per 1,000 recreators.

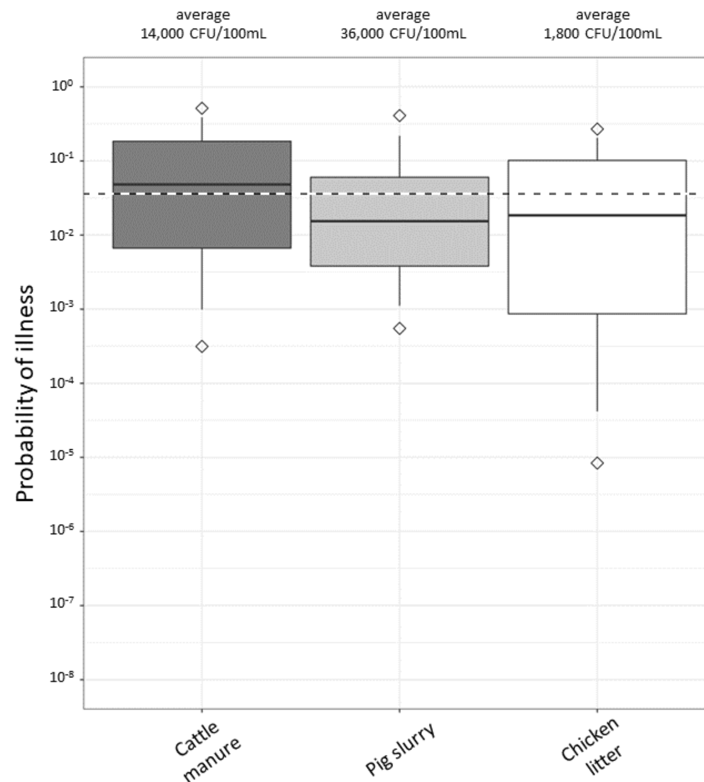


Figure 3-5. Comparison of illness risks from exposure to undiluted runoff from land-applied manures from three different species (from Soller et al. [2015]).

The results from this example forward QMRA show that cattle manure is associated with the highest risk and pig slurry with the lowest risk among the three nonhuman fecal sources within this exposure scenario. In all three scenarios, enterococci densities were substantially elevated compared to the 2012 RWQC recommendations; however, for the pig and chicken scenarios, risks were well below the target illness rate associated with the 2012 RWQC. Although risks in the cattle scenario were higher than the EPA's recommended target illness rate, the risk occurred at a much higher enterococci GM. The interpretation of the results of the forward QMRA is an important aspect of this TSM. The results from the example are also discussed below in Section 3.3.3 to illustrate a decision point on proceeding to Step 4.

3.3.2 Forward QMRA Approach 2: Using a Combination of Collected and Literature-based Data

Approach 2, like Approach 1, is a forward QMRA; the difference between Approach 1 and Approach 2 is how the pathogen densities are derived for input into the QMRA and how the pathogen densities are adjusted for abundance in the source and human infectivity. In Approach 2, pathogen density for each reference pathogen is estimated by adjusting literature-based data on pathogen density in the source by the proportion of measured FIB density (from Step 2) and literature-based data on FIB density in the source (See Section 2.2.2.4.3 Eq. 2A). This calculation provides an upper bound for the level of pathogens in the water because pathogen attenuation via environmental fate and transport is not directly considered. The estimated pathogen densities, like Approach 1, are then input into a forward QMRA to estimate human health risk from recreational exposure.

The prevalence and abundance of reference pathogens in selected animal fecal waste can be found in Table 2-2 (Section 2.1.1.1). Densities of FIB in selected animal fecal waste can be found in Table 2-7 (Section 2.1.2.1). Data for other animal source(s) can be found by searching the scientific literature using database searches, such as Google Scholar, PubMed, and other literature databases, including subscription-based services. To account for a mixed fecal source scenario, Schoen et al. (2011) describe an approach to adjust the proportion of FIB in a waterbody that is attributable to a specific source. This term (p_{FIB}^{S}) is included in Equation 2A. The sanitary survey supports the estimation of each source contribution, thus an estimate of FIB from each source can be developed. For example, if the sanitary survey and/or fecal source identification from the water quality study indicates that less than 10% of the fecal loading is from human sources and the rest (90%) is likely from avian and predominantly seagulls, then the calculation described below is performed once for the human source (proportion = 0.1) and once for the gull source (proportion = 0.9). The proportions of all the sources equal 1 (100% of FIB accounted for). If the sanitary survey indicates that there are multiple sources of FIB, those sources can be grouped together if the source with the highest potential levels of each reference pathogen is used as the representative group for that source. For example, if avian species are known to be the major source of FIB and there is a mixture of wild birds and chickens in the watershed, users can combine the avian sources together from a loading perspective and choose the reference pathogen and FIB data from the subsource with the highest density. Selecting the highest density is consistent with a health-protective approach for the Approach 2 analysis. If a single source is determined to be a predominant loading of nonhuman fecal waste, then this term can be considered equal to “1.0.”

Please note that the FIB data from Step 2 could be collected close to where the source is thought to contaminate surface water and under representative conditions when the source is thought to mobilize to surface water. If multiple sources are identified, having data collected proximal to the source loadings and data collected from a study site downstream can support the estimation of source contribution.

Once the density of each reference pathogen is determined, the user can proceed with the calculations for dose, probability of infection, and probability of illness. The output of Equation 2A is used in Equation 2B to calculate the dose for each reference pathogen. The calculation of dose in Approach 2 is slightly different than in Approach 1. In Approach 1, there is multiplication of pathogen density by ingested volume of water. In Approach 2, Equation 2B accounts for the density of pathogens by including the fraction of pathogens that are infectious to humans (Section 2.1.1.1, Table 2-4) and the prevalence of animals that harbor the pathogen (percentage infected) (Section 2.1.1.1, Table 2-3). The volume of water incidentally ingested will be the same as used for Approach 1. The subsequent steps of dose response and infections that lead to illness are the same in Approaches 1 and 2. Users can refer to the description of Approach 1 above for these steps of the forward QMRA.

The example forward QMRA R code in Appendix F performs forward QMRA for a hypothetical waterbody that contains a 100% gull fecal source. The parameters described above (from Tables 2-2, 2-3, 2-4, and 2-7) for gulls are used in the R code in Appendix F. The dose-response relationships (Table 2-5) and probability of infection leading to illness (Table 2-6) for *Campylobacter* and *Salmonella* are included in the R code in Appendix F. Users supply a spreadsheet of input data that contain enterococci densities in CFUs per liter (L). The estimated mean and median NGI per 1,000 recreators are outputs.

3.3.3 Interpreting Results

Sanitary survey information and water quality data were used to conduct a QMRA estimating risk at the study location receiving fecal contamination from one or more sources. The output from the forward QMRA analyses can be used to address the following question: “What is the potential human health risk from recreational exposure to water at the study location contaminated by the fecal source(s) in the watershed?” The transparent comparison of the QMRA results to current water quality recommendations and target illness rates or the current WQS constitutes an important decision point. Understanding how the results compare to the EPA recommendations will inform the decision about whether to move forward with deriving alternative criteria.

In this section, the results of the QMRAs from Approaches 1 and 2 and from the water quality distribution will be compared to the EPA’s 2012 recommendations for water quality and target illness rate. Table 3-1 outlines the different steps that can be taken based on the results of the comparison. Note that the case where the water quality level at the study location is below the recommended water quality is included in Table 3-1 for completeness. Although any location could be evaluated for alternative criteria, the EPA expects that most sites having water quality levels below current recommendations would not be evaluated for alternative criteria; instead, the EPA-recommended criteria or the current applicable WQS would apply. Waters affected by secondary-treated and disinfected wastewater effluent are a notable exception to this point. WWTP effluent-affected waters represent a condition where water quality can meet the recommended criteria; however, elevated illness can exist in downstream waters due to the differential treatment efficacy between culturable FIB and pathogens such as enteric viruses and protozoa. This combination of outcomes is beyond the scope of this TSM because this document focuses on adjusting the 2012 RWQC water quality values for less risky nonhuman fecal sources. However, the same tools described in this TSM (e.g., sanitary characterization and QMRA) could be used to further characterize risk in high-risk scenarios but are not the focus here. At this point in the TSM process, it is assumed that waters affected by substantial inputs of human fecal contamination, such as treated human wastewater effluent, are not being considered for alternative culturable FIB-based criteria based on predominantly nonhuman fecal sources.

Table 3-1. Comparing forward QMRA and water quality monitoring results to the EPA’s 2012 RWQC.

Relationship of water quality and target illness recommendations	Monitoring results are:	
	< Recommended GM	> Recommended GM
QMRA results < target illness rate	National RWQC or applicable WQS may apply, or alternative criteria could be derived if waterbody is predominantly affected by nonhuman fecal sources (Go to Step 4).	Go to Step 4.
QMRA results > target illness rate	Beyond the scope of this TSM.	National RWQC or applicable WQS may apply. Relative QMRA if nonhuman fecal sources predominate.

Together, the results from Approaches 1 and 2 above can result in a range of estimated risks (i.e., the range between the median estimates) for a given nonhuman fecal source. The range results from the assumptions, including pathogen loading and recreational exposure, applied in each approach. For example, pathogen densities in water can be characterized using monitoring data from a waterbody some distance downstream of a source or from water upstream near where the source is thought to enter the water. Although the EPA recommends that monitoring data reflect the hazardous condition that results in the source entering the water, pathogen loadings can be associated with variability, and monitoring results below the LOQ of the enumeration method can occur. Approach 2 does not account for pathogen fate and transport and can be considered a "direct deposition" scenario. It is expected that Approach 2 results could be at the higher end of potential risk from the nonhuman fecal source. Taken together, QMRA results based on data from both Approaches 1 and 2 provide valuable insight for understanding the potential human health risks in the watershed.

There are three key outcome categories to consider for a range of risk estimates resulting from Approaches 1 and 2 compared to the EPA's recommended target illness range: (1) the range is wholly below the EPA's recommended target illness level; (2) the range straddles the target illness level, or (3) the range is wholly above the target illness level. When the range is below, users can proceed to Step 4. If the comparison of results indicates "Go to Step 4" (Table 3-1), and the other lines of evidence from the sanitary characterization support predominantly nonhuman fecal sources, proceeding to Step 4 to calculate a GM value that corresponds to the target illness rate can be substantiated.

As shown in Figure 3-5, the hypothetical waterbodies with pig and chicken fecal sources have enterococci levels (36,000 enterococci CFU per 100 mL and 1,800 enterococci CFU per 100 mL for pigs and chickens, respectively) greater than the RWQC recommended GM of 35 enterococci CFU per 100 mL. Looking at Table 3-1, they are in the column where monitoring results are "> Recommended GM." Next, the predicted illness rates are 15 illnesses and 18 illnesses per 1,000 recreation events for pig- and chicken-impacted waterbodies, respectively. These predicted illness rates are below the RWQC associated target illness rate of 36 NGI per 1,000 recreators (row in Table 3-1: "QMRA results < target illness rate"). Considering both pieces of information—waterbody FIB GM and predicted illness level— and referring to Table 3-1, these two nonhuman source scenarios fall in the upper right quadrant of the table. For these two scenarios, the user could proceed to Step 4 of this TSM to derive criteria.

When the range straddles the recommended target illness level, users can apply their best professional judgment to determine whether to go directly to Step 4 or decide to perform a relative QMRA. For example, if the recommended target illness rate is relatively close to the upper end of the estimated range, then the decision to move on to Step 4 is based on expert judgment. Approach 2 contains health-protective assumptions that may overestimate actual risk at a specific location. If the target illness rate is closer to the bottom end of the estimate risk range, users can decide to conduct the relative QMRA before going on to Step 4 to provide substantiation that the waterbody would be protective at the EPA's recommended level of water quality.

If a comparison of results demonstrates both water quality and estimated illness are above the recommended GM and the associated target illness rate, then there are two choices:

1. Apply the EPA's RWQC recommendation or the applicable WQS.
2. If the evidence collected indicates that nonhuman fecal sources predominate, then a relative QMRA could be conducted to understand what the estimated target illness rate would be if the waterbody met the EPA's recommended criteria value.

As shown in Figure 3-5, the hypothetical waterbody with cattle as a fecal source has enterococci levels greater than 14,000 CFU per 100 mL compared to the RWQC recommended GM 35 enterococci CFU per 100 mL. Looking at Table 3-1, this waterbody falls in the column where monitoring results are "> Recommended GM." The predicted illness rate for the cattle-impacted simulated waterbody is 46 NGI per 1,000 recreators, above the RWQC target illness rate of 36 NGI per 1,000 recreators. This corresponds to Table 3-1 row: "QMRA results > target illness rate." Taking both these pieces of information—waterbody FIB GM and predicted illness level—and referring to Table 3-1, this example cattle-impacted waterbody falls in the lower right quadrant of the table. In this situation, the TSM user can apply the EPA's recommended RWQC, or a relative QMRA can be conducted to determine if the waterbody met the EPA's target illness rate at the RWQC recommended GM (in this case, 35 enterococci CFU per 100 mL).

A relative QMRA complements a forward QMRA by estimating risks from specific fecal sources at a specified FIB density (Soller et al., 2015). The specified FIB density would be one of the EPA-recommended values, so this analysis estimates the potential risk for the nonhuman source relative to the EPA's RWQC. The relative QMRA process is diagrammed in Section 2.2.2.4.4. It addresses the following question: "What would the predicted illness level be if the waterbody water quality was at the EPA's recommended criteria value (e.g., 35 enterococci CFU per 100 mL)?" For example, if monitored water quality results from the study location is a GM of 1,000 enterococci CFU per 100 mL and the QMRA-estimated median illness level is 40 NGI per 1,000 recreators, and assuming the choice of target illness rate was 36 NGI per 1,000 recreators, the relative QMRA step would predict the illness level for the waterbody studied if the GM were 35 enterococci CFU per 100 mL.[24] This comparison can demonstrate whether the waterbody is protected at the EPA-recommended target illness rate. If the relative QMRA step demonstrates that the predicted illness rate is below the EPA's recommended target illness rate, users can decide to proceed to the next step. Note that the results for the forward QMRA where both water quality and estimated illness are above the EPA recommendations may indicate that the study site characteristics and/or conditions could be substantially affecting water quality and risk estimates, which also may limit the generalization of derived alternative criteria to other locations where nonhuman sources predominate. In the example discussed above (Figure 3-5), pathogen mobilization was induced via simulated rainfall at a rate of 6.75 centimeters (cm)/hour (Soller et al., 2015). These conditions may not be reflective of other locations.

The example discussed above in Section 3.3.1 (Soller et al., 2015) includes a relative QMRA step for cattle, pig, and chicken fecal sources, and it is useful for illustrating relative risk from nonhuman sources compared to the EPA's RWQC recommendations. In this example, the probability of illness for

[24] The recommended health goal associated with the national 2012 RWQC is 32 or 36 NGI per 1,000 primary contact recreation events. Enterococci at 35 CFU per 100 mL is associated with 36 NGI per 1,000 primary contact recreation events. Recreators are those who recreate on or in the water.

each source is expressed in terms of the FIB (*E. coli* or enterococci) from each source to the target illness rate associated with the EPA's RWQC (Figure 3-6). Note that in the forward QMRA, each source was associated with FIB levels well above the RWQC recommendations, although the risk of illness was lower for pigs and chickens. In Figure 3-6, the risk of illness from each source is compared as if the water were meeting the RWQC. Although QMRA typically focuses on risk from exposure to a pathogen,

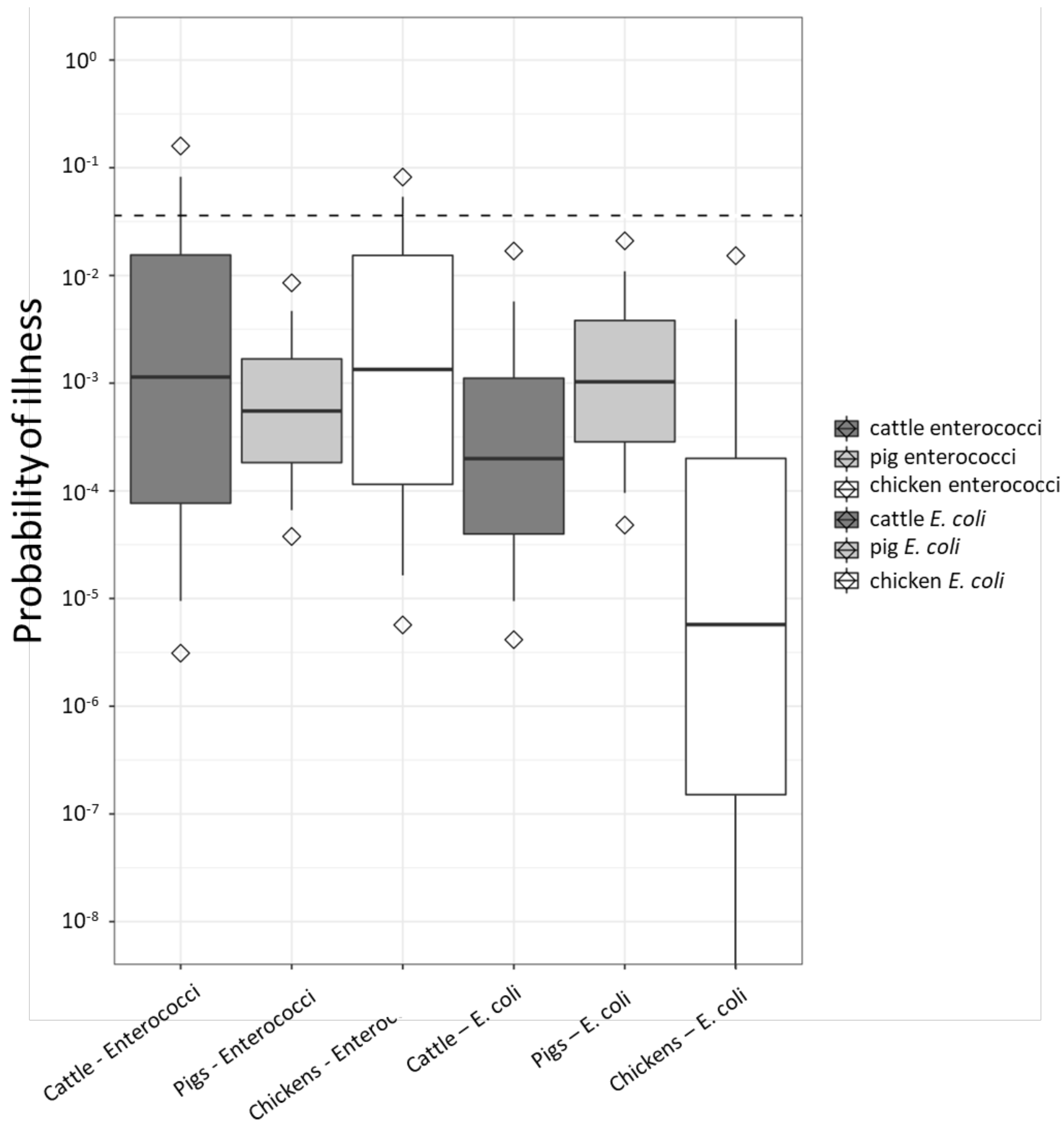


Figure 3-6. Box and whisker plot displaying the relative QMRA probability of illness across fecal contamination from different species (from Soller et al. [2015]). The line in the middle of each box is the median. The upper and lower edges of each box are the 75th and 25th percentiles. The bars (whiskers) extend to the 90th and 10th percentiles. The diamonds are at the 95th and 5th percentiles. The dotted line represents the EPA's recommended target illness rate of 36 NGI per 1,000 recreators.

expressing the risk in terms of FIB levels is helpful for comparing to the EPA's recommendations and/or existing state WQS. For a waterbody affected by cattle fecal material, the predicted illness rate is 30 times lower (~1.1 NGI per 1,000 recreators) compared to the EPA target illness rate. For a pig-impacted waterbody, the predicted illness rate is 65 times lower (~0.5 NGI per 1,000 recreators) compared to the EPA target illness rate. For a chicken-impacted waterbody, the predicted illness rate is 25 times lower (~1.3 NGI per 1,000 recreators) compared to the EPA target illness rate (Soller et al., 2015).

Given the results of the relative QMRA, namely that the predicted level of illness for the hypothetical cattle-impacted waterbody is 1.1 NGI per 1,000 recreators (if the enterococci levels were at 35 CFU per 100 mL), this example could proceed to Step 4 of the TSM. It meets the condition that the predicted level of illness is below 36 NGI per 1,000 recreators. If the predicted level of illness had been above 36 NGI per 1,000 recreators, then users are advised to apply the nationally recommended RWQC. In this case, users may want to consider characterizing another nonhuman fecal source-affected waterbody for the development of alternative WQC.

3.4 Step 4: Derive Site-Specific Alternative Criteria

Text Box 3-6. Decision Points Step 4

Choose one of the EPA's target illness levels or a lower level. The magnitude, duration, and frequency are scientifically defensible and protective of the use.

Step 4 describes the derivation of alternative WQC (i.e., GM, STV, and BAV) that adjust the EPA's 2012 RWQC for waters where nonhuman fecal sources predominate. Users need to choose one of the two illness rates recommended by the EPA to support the derivation (Text Box 3-6). Examples presented in Step 4 incorporate the 36 NGI per 1,000 recreators target illness rate because this benchmark facilitates comparisons to many existing state WQS. The methods and approaches discussed in Step 4 (see Figure 3-7) would apply equally well if the 32 NGI per 1,000 recreator rate was selected.

The target illness rate chosen is a policy decision that should be clearly described in the documentation for the WQS submission. When evaluating WQC submissions, the EPA may return submissions to request additional information or to disapprove submissions that do not document sufficient detail (40 Code of Federal Regulations [CFR] 131.5 and 131.6) or scientific rationale.

Most commonly, QMRAs are conducted using microbial density as an input, and the output is the estimated risk level, as was discussed for Approaches 1 and 2 in Step 3. In contrast to a forward QMRA, a "reverse" QMRA considers associations in the opposite direction by starting with a target illness rate and then calculating a microbial density. The same QMRA parameters and values are used as described above in Step 3: (1) the volume of water ingested during swimming; (2) the density of pathogens in the specific sources; (3) the mathematical dose-response relationships and the parameter values for each reference pathogen; and (4) the conditional probability of illness given infection for different pathogens. Reverse QMRA is discussed in more detail in Section 2.2.2.4.5.

The EPA and others have conducted and documented QMRAs for scenarios consisting of waters affected by human, cattle, pig, chicken, gull, and nonpathogenic fecal sources, and other nonhuman and mixed fecal discharges to a watershed (U.S. EPA, 2010a; Schoen and Ashbolt, 2010; Soller et al., 2010a,b, 2014, 2015, 2016; Schoen et al., 2011; McBride et al., 2013). Some QMRA analyses have considered single nonhuman sources to estimate relative risks compared to human sources (U.S. EPA,

2010a; Soller et al., 2010). Other QMRAs have included mixed fecal contribution scenarios consisting of human and nonhuman fecal sources (Schoen and Ashbolt, 2010; Schoen et al., 2011; Soller et al., 2014). Mixed source contributions in a watershed may be the more commonly encountered scenario. An important result noted in the QMRAs evaluating mixed fecal sources was that the risks are determined predominantly by the proportion of the contamination source with the greatest ability to cause human infection, not necessarily the source that contributes the greatest number of FIB (Schoen et al., 2011; Soller et al., 2014).

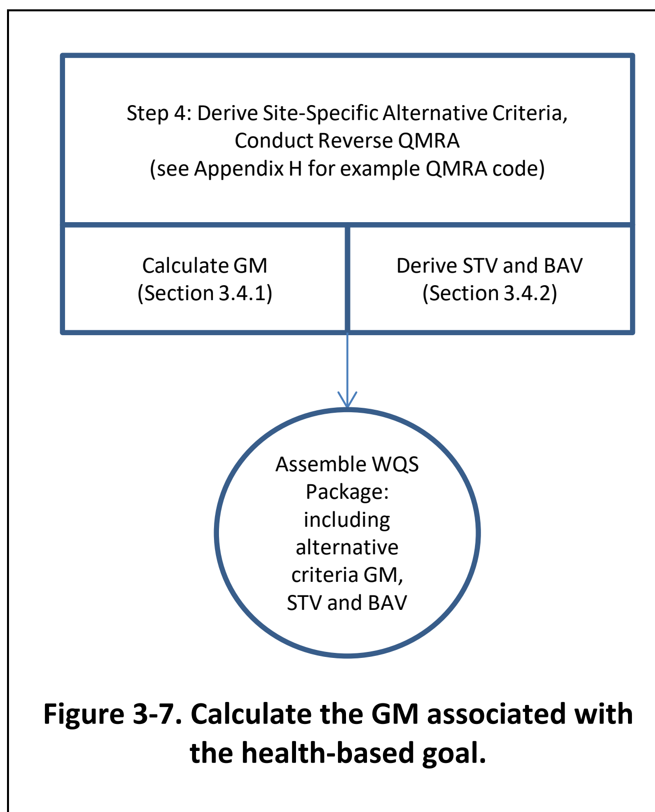


Figure 3-7. Calculate the GM associated with the health-based goal.

When human sources are a component of the mixed fecal loading, the viral pathogens in human waste can pose the highest potential of gastrointestinal illness to recreators. For example, Soller et al. (2014) simulated the influence of multiple sources of culturable enterococci in a recreational water on the illness potential those sources present. Combinations of human and nonhuman fecal sources were considered and varied from zero to 100% of the mixture. Results demonstrated that the estimated risks of gastrointestinal illness from recreational exposures to fecal mixtures were up to 50% lower for mixtures with an approximately 30% human component compared to fecal loadings of 100% human as measured by enterococci (Figure 3-8). In this example, gull, pig, or chicken sources with a low human contribution (i.e., less than the approximate 30% contribution) resulted in numerically higher, but equivalently protective, enterococci levels at the 36 NGI per 1,000 recreators target illness rate. Nonpathogenic sources include autochthonous enterococci and *E. coli* (Byappanahalli et al., 2006).

Please note that the results presented in Figure 3-8 represent reverse QMRA results based on the information available when the analysis was conducted. The information included parameter values, some of which have been updated as newer science has become available (e.g., dose responses for norovirus and other enteric pathogens and incidental ingestion data). Figure 3-8 is presented as a demonstration based on data from three nonhuman sources of higher FIB values possible at the same target illness rate as the EPA recommends. Note the trend of decreasing enterococci values as the human contribution increases relative to the nonhuman contribution. Results would support a science policy decision of approximately 30% human contribution (i.e., FIB values at this percentage are approximately a half log higher than at 100% human) as an inflection point above which risks from human sources predominate the risk profile.

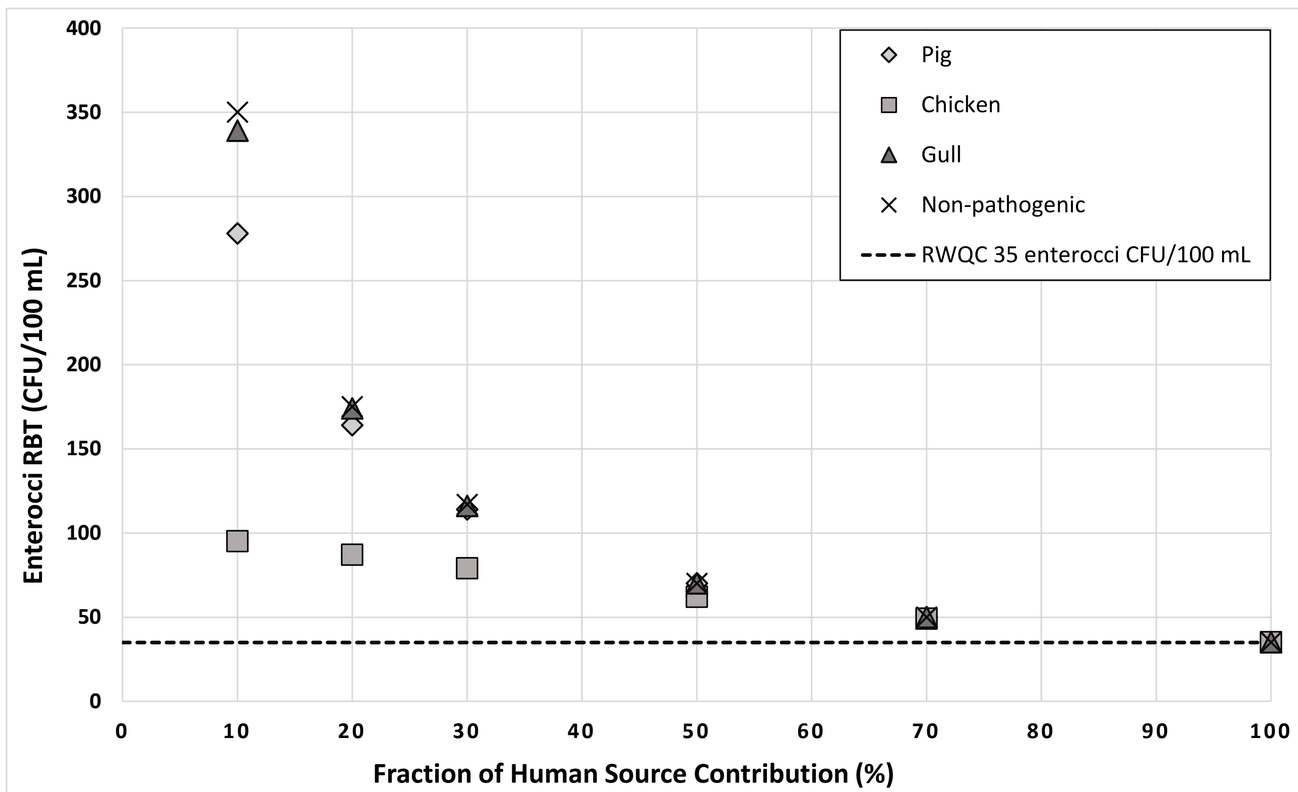


Figure 3-8. Predicted GM enterococci densities corresponding to 36 NGI per 1,000 recreators for waters impacted by mixed sources of fecal contamination for three animal species (CFU per 100 mL) (adapted from Soller et al. [2014]). The output from the reverse QMRA is a FIB density that can be used as a GM corresponding to the target illness rate and is called a RBT.

Table 3-2 presents selected results shown in Figure 3-8 to help support the interpretation of the effect of mixed fecal sources (e.g., nonhuman and human fecal contributions) affecting a waterbody. In this example, the susceptibility of a waterbody to human fecal contamination is “binned” into low, medium, and high susceptibility, representing human contributions of 33% or less, 34%–67%, and 68% or greater, respectively. Due to the potential variability associated with estimating levels of FIB based on monitoring, the “medium” and “high” bins are considered to be similar to the human-dominated scenario underpinning the 2012 RWQC recommended criteria. Waterbodies where human contributions represent less than a third of the total fecal loading fall into the “low” human fecal susceptibility bin.

When states decide to adopt new or revised WQC into their WQS, the criteria must be scientifically defensible and protective of the designated uses of the waterbodies. The EPA’s regulation 40 CFR §131.11(b)(1) provides that “In establishing criteria, states should (1) Establish numerical values based on (i) 304(a) Guidance; or (ii) 304(a) Guidance modified to reflect site-specific conditions; or (iii) Other scientifically defensible methods.”

Include the same basic elements as the national RWQC—magnitude, duration, and frequency—for the alternative criteria developed using this TSM. Magnitude is the numeric expression of the maximum amount of the pollutant that may be present in a waterbody that supports the designated use. Duration is the period of time over which the magnitude is calculated. The frequency of excursion describes the maximum number of times the pollutant may be present above the magnitude over the specified time period (duration). A criterion is set in a WQS such that the combination of magnitude, duration, and frequency protects the designated use (such as primary contact recreation).

Table 3-2. Predicted GM enterococci densities (CFU per 100 mL) in mixed sources of fecal contamination that correspond to 36 NGI per 1,000 recreators. Values presented are for illustrative purposes and are not meant to be used as alternative criteria.

Nonhuman source of fecal contamination	Susceptibility of waterbody to human fecal contamination (percent human contribution)				
	Low			Medium	High
	≤ 10%	≤ 25%	≤ 33%	34%–67%	> 67%
Nonhuman source #1[a]	95	83	76	Nationally recommended RWQC apply	
Nonpathogenic source[b]	350	140	106		

Notes:

a. Nonhuman fecal source examples with relative risk evaluations reported in the peer-reviewed scientific literature include cow, chicken and pig manures, bird/gull feces, and mixed human and nonhuman fecal contributions. Contributions can be “fresh” and directly deposited in a water body, deposited on land due to livestock grazing, or may be aged manures applied at agronomic rates to fields.

b. Nonpathogenic sources include autochthonous or other sources of enterococci and E. coli not directly associated with the loading of feces to a waterbody.

The magnitude is expressed in terms of a GM and a STV. The EPA’s 2012 RWQC recommends expressing the criteria magnitude as a GM value corresponding to the 50th percentile of the water quality distribution and an STV corresponding to the 90th percentile of the same water quality distribution. In the RWQC, two sets of GM and STV are recommended, each associated with a specific target illness level. The GM calculated in Step 4 is related to one or the other target illness rate in the 2012 RWQC. For duration and frequency, the EPA recommends that the waterbody GM not be greater than the selected GM magnitude in any 30-day interval. The state would need to define the duration in their proposed WQS. There should not be greater than a 10% excursion frequency of the selected STV magnitude in the same 30-day interval. The EPA does not specify sampling frequency or the number of samples required. States can determine the sampling frequency and the number of samples that provide the degree of statistical confidence they want. Some resources for evaluating sample sizes include Smeets et al. (2010) and Helsel (2012).

Although states have flexibility in selecting the construct of their WQS, the submissions will need to clearly and transparently document that the standards are scientifically defensible and protective of the designated use (see Appendix D for the WQS checklist). Supporting documentation for any deviations from the EPA’s recommendations is recommended. WQS submissions adopting the EPA’s recommended construct will be easier to evaluate relative to the 2012 RWQC recommendations.

3.4.1 Reverse QMRA and Calculating the GM

As discussed in Section 2.2.2.4.5, a reverse QMRA approach is used to estimate the FIB levels for waterbodies predominated by nonhuman fecal sources, including human and animal fecal contamination mixtures, at a specified target illness rate. A reverse QMRA approach can be used to estimate the level of a pathogen that results in an estimated or observed level of illness (e.g., what level of norovirus is needed to cause the level of gastrointestinal illness reported in a waterborne outbreak). In Soller et al. (2010b), densities of each reference pathogen were computed corresponding to the illness reported on each day in the NEEAR epidemiological study, resulting in a pathogen profile for the fecal loading at the study locations. The reverse QMRA approach discussed in this TSM is computationally more involved because the relative level of FIB from each fecal source is considered in the derivation of the FIB GM anchored to the target illness level selected. An example is provided to

aid the user in conducting the reverse QMRA analysis for this step. At the end of this step, a GM for enterococci or *E. coli* that corresponds to either 32 or 36 NGI per 1,000 recreators will be derived.

3.4.1.1 Reverse QMRA modeling description and assumptions

This TSM provides a case study that simulates a hypothetical scenario of a waterbody affected by a mixture of gull and human fecal sources in different proportions (Appendix G). In this scenario, a reverse QMRA analysis is conducted to derive an alternative FIB GM and is presented as an illustrative example of the process described in this TSM. The gull and human fecal inputs are varied in proportions between 10% and 100% human. The reverse QMRA case study is summarized in this section, with full documentation described in Appendix G and the associated reverse QMRA Python code provided in Appendix H.

The reverse QMRA uses the following inputs to derive a GM for enterococci anchored to a recommended target illness rate for each of the fecal source mixtures:

- Target illness rate: A target illness rate of 36 NGI per 1,000 recreators was recommended in the 2012 RWQC (U.S. EPA, 2012).
- Ingestion volume: A median general population ingestion volume of 19 mL per recreational event (Dufour et al., 2017).
- Human fecal contamination: Norovirus was selected as the primary index pathogen. Norovirus and enterococci levels in human sewage are literature-based (Table 2-2).
- Gull fecal contamination: *Campylobacter jejuni* and *Salmonella* were selected as reference pathogens. Pathogen and enterococci levels in gull feces are literature-based (Table 2-2).
- Dose-response: The infectivity of the three reference pathogens was characterized by mathematical dose-response relationships (Table 2-5). The probability of infection leading to illness was characterized as a constant for *Salmonella* and using a hazard model function for *Campylobacter* and norovirus (Table 2-6).
- Fecal Source Apportionment: The percentage of enterococci contributed by human fecal sources was modeled separately at 10%, 20%, 25%, 30%, 33%, 50%, 60%, 67%, 75%, 90%, and 100% to reflect a range of possibilities providing context for the results.
- Simulated human mixture: To anchor the reverse QMRA to the 2012 RWQC a simulated human source was created that provides model results of 35 enterococci CFU per 100 mL at the illness level of 36 NGI per 1,000 recreators. The simulated human mixture is comprised of secondary treated effluent with some raw sewage. In this mixture, the effluent contributes pathogens (i.e., risk of gastrointestinal illness), and the raw sewage primarily contributes the FIB (See Schoen et al., 2011 and Soller et al., 2014 for more details).

Generally, a reverse QMRA begins with a target illness level and ends with the quantity of stressor associated with the input target illness level. In this reverse QMRA, the pathogen (stressor) density is linked to an enterococci level, so the output is a GM density of enterococci that is associated with the input target illness rate. Equations 3A through 3L found in Section 2.2.2.4.5 are used to combine the information in the bullets above. Conceptually the reverse QMRA is shown in Figure 2-10. An initial

enterococci density estimate is provided to start the iterative process of calculating the FIB level associated with the target illness level, which is shown as the loop between the diamond and the beginning of the model in Figure 2-10. The density of each pathogen in the human or nonhuman source is used to compute a dose. The dose-response functions are used to compute the probability of infection, given the dose and a recreational exposure, which is then used to compute an illness rate based on the probability of infection leading to illnesses. The computed probability of illness is then compared to the target illness rate. If they do not match, then the process is repeated in an iterative fashion until the computed illness rate matches the input target illness rate. Once the computed illness rate matches the target illness rate, the corresponding enterococci density is considered the RBT for enterococci CFU per 100 mL.

The reverse QMRA Python code in Appendix H is annotated so users can follow the purpose of each line of code.[25] Briefly, the Python code begins with defining the target illness rate and the ingestion volume point estimate. The input values of the density of the enterococci for each source are then assigned to variables in the code (as shown in Appendix G: Table G-3). Input values for the prevalence of each pathogen in each source are assigned in the next section of the code, followed by the fraction of infectious pathogens in each source. The structure of the reverse QMRA requires iterative solving, which is where an initial value of FIB density corresponding to the target risk is estimated and used by an iterative formula that specifies the mathematical relationship between FIB density and risk consistent with the input assumptions and generates a sequence of improving approximate solutions for the FIB RBT. In the case study example, an enterococci level of 50 CFU per mL (500 CFU per L in the code) was provided as an initial estimate of the enterococci density corresponding to the target risk rate for each source. This value is refined by computer code through numerical iteration to derive the specific enterococci density for each source and at each simulated human source contribution level, resulting in 11 fecal mixture proportions (i.e., model convergence). The code includes an internal forward QMRA to confirm that the model has reached convergence for each scenario.

The output of the reverse QMRA Python code (Appendix H) is a table that contains enterococci densities (CFU per 100 mL) for the 11 apportionment scenarios for each of the four sources included in the code (gulls, swine, chicken, and nonpathogenic). The reverse QMRA code example implements TSM Equations 3A through 3L found in Section 2.2.2.4.5.

Figure 3-9 displays a graphic representation of the results from the reverse QMRA presented in Appendix G. Each point represents the enterococci GM corresponding to the 36 NGI per 1,000 recreator target illness rate for the various proportional gull and human fecal mixtures. The dotted line on the graph represents 35 enterococci CFU per 100 mL. At 100% human contribution, the model output is anchored at 35 enterococci CFU per 100 mL and 36 NGI per 1,000 recreators. As the percentage of human sources decreases and the percentage of gull fecal sources correspondingly increases, the level of enterococci that corresponds to the target illness rate increases. The hypothetical waterbody with 10% human fecal source and 90% gull source results in 350 enterococci CFU per 100 mL at the recommended target illness rate.

[25] The python code in Appendix H also includes the parameter information for swine and chicken fecal sources (results not shown).

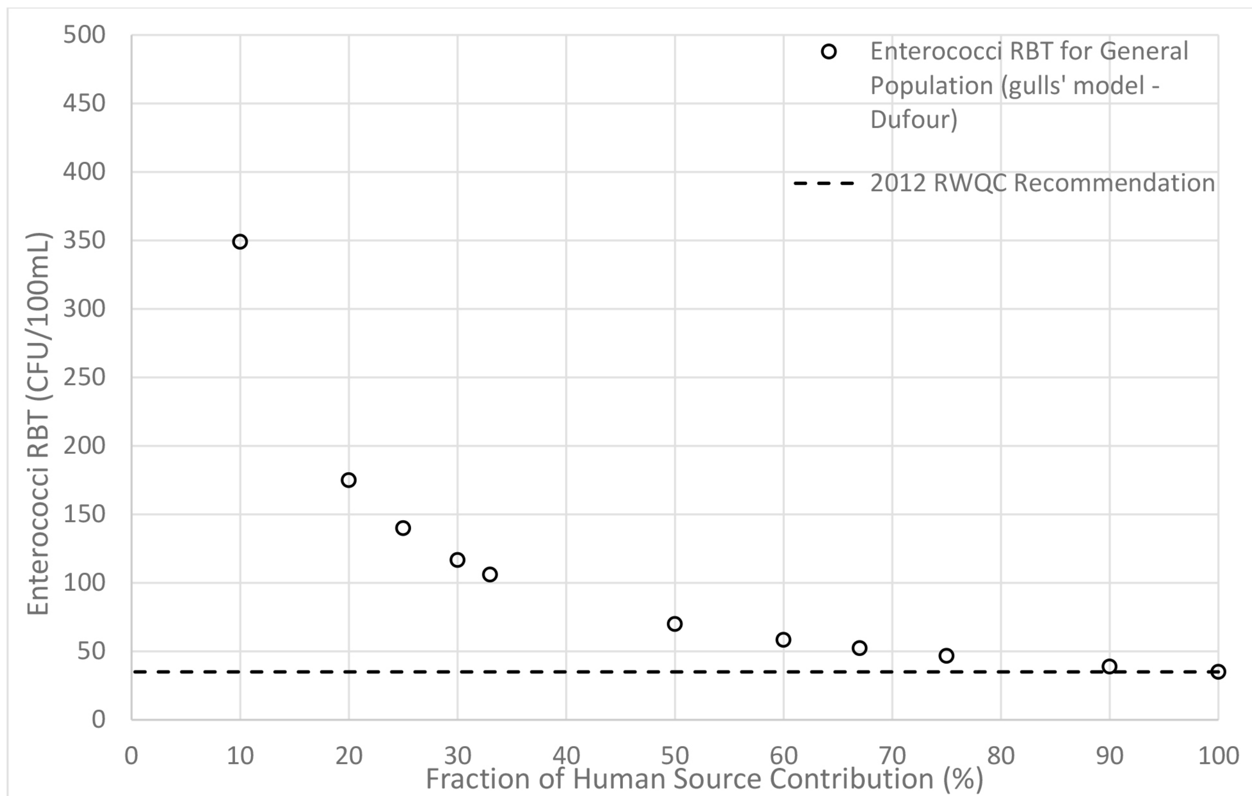


Figure 3-9. Reverse QMRA results for the gull case study. Each data point on the graph represents the enterococci GM associated with the 36 NGI per 1,000 recreator target illness rate for varying proportions of gull and human fecal mixtures.

Table 3-3 presents the threshold enterococci level corresponding to the target risk level (36 NGI per 1,000 recreators) for two different source mixtures (human-gull mixture and human-nonpathogenic mixture) considered in the low susceptibility of human influence (see Table 3-2 for more detail). The gull row shows the numerical values for three of the points in Figure 3-9 above. The nonpathogenic source was not plotted in Figure 3-9 because the points overlap the gull results. The levels of human contribution in Table 3-3 were selected because these levels are conceptually straightforward to visualize and estimate based on the qualitative and quantitative information gathered as part of the Sanitary Characterization (Step 1, Section 3.1, and Step 2, Section 3.2). Additionally, a one-third proportion of human fecal loading results in an approximate half log increase in the GM enterococci value, while a 10% human loading corresponds to a one log increase in the GM enterococci value.

The values presented in Table 3-3 represent the output from the reverse QMRA code using the data inputs identified above providing a GM threshold for the hypothetical waterbody associated with one of the EPA's recommended target illness rates. In this example, if the sanitary characterization identified that the waterbody was receiving fecal loading that was predominantly gull, with a quarter of the loading from humans, an enterococci GM value of 140 enterococci CFU per 100 mL would correspond to the chosen target illness rate. The enterococci values in Table 3-3 illustrate the relative risk difference between gull and human fecal inputs but should not be interpreted as criteria recommendations for a specific nonhuman source. These values are provided as examples only.

Table 3-3. Enterococci values corresponding to the target illness rate for selected nonhuman and human fecal mixtures.

Nonhuman source	Human source contribution[a]		
	≤ 10%	≤ 25%	≤ 33%
Gull	349	140	106
Nonpathogenic	350	140	106

Notes:
a. The three percentages of human source contribution are associated with a low susceptibility for human fecal influence. Output from Case Study Example in Appendix G, enterococci RBT (CFU/100 mL) corresponding to the target illness rate.

To complete criteria derivation, the STV, duration, and frequency components are also needed. Calculation of the STV and BAV corresponding to the GM is discussed in Section 3.4.2 below. In this example, the STV and BAV values corresponding to an enterococci GM of 140 CFU per 100 mL are 513 (or could be rounded to 510) and 277 (or could be rounded to 280) enterococci CFU per 100 mL, respectively (Appendix G).

3.4.2 Derive STV and BAV

STV. Calculating the STV requires having the logSD[26] of the data for the indicator in the waterbody. The EPA based the 2012 RWQC STV values on the logSDs of FIB they observed during the EPA epidemiological studies. The STV represents the 90th percentile of the water quality distribution centered on the recommended GM values, so the frequency of samples above the STV can be up to 10%, and the waterbody being measured is still considered in attainment. At the NEEAR study sites, the pooled estimate for the logSD of culturable enterococci was 0.44 CFU per 100 mL (U.S. EPA, 2012).[27] The EPA used the following equation to calculate the STV at the 90th percentile for the corresponding GM value for enterococci or *E. coli* in the RWQC:

- Assuming a logSD[28] of 0.44, Equation 4A gives the 90th percentile of the distribution of a waterbody with a GM:

$$STV = \text{antilog}_{10}\,[\log_{10}(GM) + Z_{90} \times 0.44] \qquad \text{[Eq. 4A]}$$

Where:

STV is the statistical threshold value.

Z_{90} = 1.28 is the 90th percentile of the standard normal distribution (unitless).

GM is the geometric mean of the waterbody in units CFU per 100 mL.

0.44 (units enterococci CFU per 100 mL) is the logSD of the data.

[26] The logSD is the SD of the base 10 log of the data. The antilog of the logSD would be the geometric SD.
[27] The EPA's 2012 RWQC page 40.
[28] The EPA based the SDs on water quality data from the EPA epidemiological studies. The logSD for the 1986 freshwater *E. coli* studies was 0.40 CFU per 100 mL; in the NEEAR studies, using the marine and freshwater beaches combined (not including Surfside or Boquerón), the logSD for culturable enterococci was 0.44 CFU per 100 mL.

Different waterbodies can have different levels of water quality variability. Use either the logSD that the EPA used in the RWQC or calculate the logSD for the FIB data collected in Step 2. The logSD can be calculated using various software packages, including MS Excel. The first step is to log-transform the data collected. In Excel, for example, use the "STDEV" or "STDEV.S" functions to calculate the SD of the log-transformed values. This value can replace the 0.44 value in the above equation.

BAV. The EPA provided recommended BAVs, which correspond to the 75th percentile of the water quality distribution. The BAV is calculated using the same SD used to calculate the STV. BAVs are not used for determining use attainment. The BAV provides a decision point for beach managers based on a single monitoring sample result for use in beach notification programs. Recreational exposure risks are not linked to, nor predicted by, any single monitoring sample results, so the BAV represents a precautionary tool on any given day when the GM is unknown. Because the BAV represents the 75th percentile, water quality can be expected to exceed this value 25% of the time and the waterbody being monitored would still be attaining the use.

- Assuming a logSD of 0.44, Equation 4B gives the 75th percentile of the distribution of a waterbody with a GM:

$$BAV = \text{antilog}_{10}\left[\log_{10}(GM) + Z_{75} \times 0.44\right] \qquad \text{[Eq. 4B]}$$

Where:

STV is the statistical threshold value.

$Z_{75} = 0.675$ is the 75th percentile of the standard normal distribution (unitless).

GM is the geometric mean of the waterbody in units CFU per 100 mL.

0.44 (units enterococci CFU per 100 mL) is the logSD of the data.

4.0 Effects Characterization

4.1 Methods to Evaluate Health-Protective Values for Different Susceptible Subpopulations

Susceptible subpopulations can be characterized by intrinsic traits (e.g., age, gender, genetic traits, immune status and other biological factors) and acquired traits (e.g., exposure, behaviors), which have the potential to influence both the exposure profile and the health outcomes in these subgroups (U.S. EPA, 2000c, 2014b). The 2012 RWQC recommendations are based on studies reporting AGI from event-based recreational exposure to ambient waters receiving human fecal contamination (U.S. EPA, 2012). Reported recreational exposures are characterized by either interviewing people who participate in recreational activities (Schets et al., 2011; Deflorio-Barker et al., 2017; Wade et al., 2006, 2008, 2010, 2022) or by estimating incidental ingestion in controlled settings (Dorevitch et al., 2011; Dufour et al., 2017). Additionally, because exposure can be influenced by behaviors, some studies have characterized time spent for various recreational activities (Deflorio-Barker et al., 2017; Ferguson et al., 2019, 2021). Adverse health outcomes following exposure to feces-contaminated recreational water are characterized in three main types of health studies: epidemiological, QMRA, and outbreak compilations. The etiologic agent(s) of disease are not often identified in epidemiological studies and outbreak reports. Typically, the risk from fecal contamination, and hence the pathogens that may be present in feces, is expressed as statistical association with a level of a nonpathogenic surrogate, such as FIB. The statistical association between illness and level of surrogate characterized by an epidemiological study is dependent on the pathogen profile present, which is dependent on the source of feces, the mass loading of feces and the dynamics of contamination (see Section 2.1).

4.1.1 Considering and Identifying Susceptible Subpopulations

Considering subpopulations is important because there are groups that are typically considered more susceptible to infection and illness, including more severe illness outcomes, compared to healthy adults: pregnant women; neonates and children; people over 65 years old; individuals residing in nursing homes or related care facilities; and cancer, organ transplant, and AIDS patients (Haas et al., 2014). Subgroup differentiation is not necessary unless there is evidence for relevant differences between the subgroups (U.S. EPA, 2014b). QMRA documentation presents a scientific rationale for dividing subgroups as well as data that directly pertain to that subgroup or could be adjusted to address that subgroup. When considering risks from pathogen exposures, factors that influence the identification of a “susceptible subpopulation” include the exposure profile, susceptibility to infection, susceptibility to illness given infection, and severity of illness. Exposure is influenced by factors such as biology and behavior. Susceptibility to infection and illness can be affected by an individual’s immune and gastrointestinal status or by a genetic host factor such as a pathogen receptor, where individuals without the receptor are not susceptible to infection and individuals with the receptor are susceptible to infection upon exposure to a specific pathogen (Nordgren and Svensson, 2019). Infection, illness, and illness severity are also influenced by the virulence, pathogenicity, and host specificity of the pathogen (U.S. EPA, 2014b).

People with compromised immune systems and/or concurrent health conditions are considered a high-risk subgroup because they can be more susceptible to infection and subsequent illness and can experience more severe illness symptoms and health outcomes, including death (U.S. EPA, 2014b). Risk communication for this subgroup regarding recreational exposure to ambient waters focuses on

preventing exposure because it is not possible to achieve a “safe” exposure to ambient water that may contain feces-associated and/or naturally occurring pathogens.

Age groups are considered as subpopulations in the context of acute risk of illness from exposure to fecal-associated pathogens because health study results published in the scientific literature demonstrate a distinct difference in exposure, illness burden and potential severity of health outcomes among broad age groupings (Arnold et al., 2016; Dufour et al., 2017; Deflorio-Barker et al., 2017; Verhougstraete et al., 2020; Wade et al., 2008, 2022). Risk differences can be influenced by one or more of the following: (1) children’s immunological, digestive, and other bodily systems that are still developing; (2) children’s greater exposure because they ingest more water and breathe more air in proportion to their body weight than adults; and (3) children’s behavior, such as increased time spent in water and more vigorous activity, that might result in increased exposure in comparison to adults. Published epidemiological and outbreak information has demonstrated that children have a higher risk of illness compared to adults when exposed to human fecal contamination in recreational waters (Arnold et al., 2016; Mosnier et al., 2018; Schets et al., 2018; Sips et al., 2020; Wade et al., 2008, 2020). Statistically significant increased water ingestion among children 6–10 years old was documented by Dufour et al. (2017). Modeling the ingestion data together with the time spent recreating demonstrated that children less than 10 years old exhibit a higher-exposure profile (Deflorio-Barker et al., 2017). The exposure data support the health study results; together, they demonstrate that children are more susceptible to gastrointestinal illness in feces-contaminated ambient recreational waters. Due to the nature of the data available, the age groupings used to characterize risk from fecal contamination are less granular than the lifestage breakouts typically considered in chemical risk assessments. In contrast to chemical contaminants in water, the adverse health effects associated with human exposure to waterborne pathogens have been best documented for event-related (i.e., short-term, single exposure) rather than chronic exposure over extended periods of time (U.S. EPA, 2014b).

4.1.2 Accounting for Differential Susceptibility

The development of the 2012 RWQC was supported by data collected during epidemiological studies that included adults and children. In the older epidemiological studies, participants under 19 years old comprised approximately 45% of swimmers (Dufour, 1984). In the NEEAR study, children aged 10 years and younger comprised approximately 17%–30% of swimmers at the beach locations studied (U.S. EPA, 2012). According to the U.S. Census data for 2009, children younger than 10 years old make up approximately 14% of the U.S. population (Census, 2010).

The 2012 RWQC recommendations are based on health data collected for the general population (i.e., all study participants). The exposure parameters described in Section 2 of this TSM are based on data from the general population, and the pathogen dose-response data was largely based on challenge studies with healthy adults. However, data documenting child-specific parameters are available. Dufour et al. (2017) and Deflorio-Barker et al. (2017) report exposure data, including incidental ingestion volumes for younger children, older children, and adults. Arnold et al. (2016) and Wade et al. (2008, 2010, 2022) report epidemiological-based health relationships for children under 10 years old. Ferguson et al. (2019) conducted an observational study focused on children ages 1 to 6 years old and their behavior patterns at recreational marine beaches in Florida and Texas. Ferguson et al. (2021) used a virtual timing device to quantify real-time, sequential micro-activity pattern data collected from videos of 120 children at four different beaches.

4.1.2.1 Exposure: Ingestion Rate Differences by Lifestage

The EPA's 2019 Recommended Human Health Recreational Ambient Water Quality Criteria (AWQC) or Swimming Advisories for Microcystins and Cylindrospermopsin (Cyanotoxin AWQC; U.S. EPA, 2019a) reviewed seven studies to evaluate recreation-associated incidental ingestion (Dufour et al., 2006, 2017; Schijven and de Roda Husman, 2006; Dorevitch et al., 2011; Schets et al., 2011; Suppes et al., 2014, DeFlorio-Barker et al., 2017). The Cyanotoxin AWQC includes graphical visualizations of ingestion volumes for different age groups, information on the length of time individuals spend in the water (duration of exposure), and a calculation of the incidental ingestion rate, which shows that children ages 6 to 10 years old have an incidental ingestion rate higher than the other age groups characterized. The results for children from the studies are briefly summarized below.

Dufour et al. (2017) used excreted cyanuric acid to measure incidental ingestion (mL/minute) in ages 6 and above and reported higher ingestion volumes among children 6 to 10 years old compared to adults. Suppes et al. (2014) used a similar measurement method as Dufour et al. (2006, 2017), with the addition of videos to estimate time in water, to evaluate the rate of water ingested by 16 children ages 5 to 17 years. They found that children, on average, ingested pool water at a higher rate than adult participants.

DeFlorio-Barker et al. (2017) combined the incidental ingestion volumes from Dufour et al. (2017) and the self-reported time spent in the water for 12 cohorts of participants of epidemiological studies to calculate the volume of water ingested per event. The results of this study corroborate other studies that demonstrate that, on average, children have higher incidental ingestion than adults when recreating.

Schets et al. (2011) reported incidental ingestion volumes and durations of recreational events for children under 15 years old. However, the study did not further divide this cohort into younger children and older children. The incidental ingestion data for children under 15 years old represent parental estimates of volumes of freshwater incidentally ingested by their children. The exposure durations were also parental estimates. The study reported higher incidental ingestion volumes, on average, for children compared to adults (Schets et al., 2011).

Although these studies used different methodologies and have limitations regarding reporting information for different age group categories, their results show a similar pattern for children incidentally ingesting water at a higher rate than adults (U.S. EPA, 2019a).

4.1.2.2 Infection and Illness

Reported health study data show that children (e.g., < 10 years old) experience more illness compared to adolescents and adults, and associations between water quality and health effects are stronger compared to adults (Arnold et al., 2016; Verhougstraete et al., 2020; Wade et al., 2022). Arnold et al. (2016) conducted a retrospective pooled analysis of 13 prospective cohorts at marine and freshwater beaches in the United States and found that gastroenteritis risk and associated health burden was higher in young children. Multiple beaches evaluated were affected by human fecal sources, but a few had little to no identified human fecal inputs (e.g., Malibu, Mission Bay, Surfside). The authors focused on incident diarrhea, defined as three or more loose or watery stools in 24 hours following exposure, as the primary adverse health outcome. Gastrointestinal illness, defined consistently with previous studies, was evaluated as a secondary health outcome. Increases in incident diarrhea were observed as the level of exposure (body immersion, head immersion, swallowed water) increased compared to

nonswimmers with a greater effect observed for children than adults. Children under 4 years old and 5–10 years old had the most water exposure, exhibited stronger associations between levels of water quality and illness, and accounted for the largest attributable illness burden.

Verhougstraete et al. (2020) developed adjusted risk difference models (excess gastrointestinal illness per swimming event) for children (< 10 years old) and nonchildren (≥ 10 years old) and compared the results of these models to those used to develop WHO's guidelines on recreational water risks. The authors used epidemiological data published in Lamparelli et al. (2015) and additional enterococci water quality data from routine monitoring at five sewage-impacted urban beaches in Brazil, polluted by stormwater runoff, partially treated sewage, and uncontrolled wastewater discharges. The risk model included a concentration-response function that is site-specific and based on enterococci (measured using culture methods) from the 1999 epidemiological study (Lamparelli et al., 2015). The risk models indicated that children less than 10 years old can have twice the risk of gastrointestinal illness than recreators greater than 10 years old. Elevated enterococci levels resulted in an excess of 96 NGI cases per 1,000 swimming children. In addition, the enterococci levels were higher in Brazil than in the primary United Kingdom study (Kay et al., 1994) that informed WHO guidelines for recreational water.

Wade et al. (2022) reported that children who participated in the NEEAR studies were at higher risk of illness associated with exposure to fecal contamination than adults, as measured by *Enterococcus* qPCR. Wade et al. (2022) analyzed epidemiological data from 13 beaches to compare illness risks and water quality, as measured with both enterococci culture and qPCR, for different age groups, including exposure levels, beach sites, and health endpoints. Children 12 and under comprised approximately 25% of the study population (total enrollment = 83,452 participants) across the various site categories. Seventy percent of children had at least some contact with water, and 27% stayed in the water for 60 minutes or more. Gastrointestinal Illness was the most sensitive health endpoint, and the strongest associations were observed with *Enterococcus* measured by qPCR at sites with human fecal contamination. Under several exposure scenarios, odds ratios for NGI among swimmers were higher among children compared to adolescents and adults. Respiratory symptoms were also associated with *Enterococcus* spp. exposures among young children at sites affected by human fecal sources, although small sample sizes resulted in imprecise estimates for these associations. The highest odds ratios were among young children at the core NEEAR sites (OR = 2.32, 95% CI = 1.33–4.06). For severe gastrointestinal illness at the core NEEAR sites, statistically significant associations were observed among children under 6 years old who stayed in the water 60 minutes or longer with *Enterococcus* qPCR CE (8.13, 95% CI = 1.92–36.2) but were not significantly associated with *Enterococcus* CFU (Wade et al., 2022).

4.1.2.3 Availability of data on other susceptible subpopulations

A discussion of susceptible subpopulations related to pathogen exposures can be found in the EPA's Microbial Risk Assessment (MRA) Tools, Methods, and Approaches for Water Media (U.S. EPA, 2014b). Subgroupings can be delineated by age, immune status, genetic background, pregnancy, nutritional status, and social, cultural, or age-related behavior traits or geographic location (e.g., environmental justice [EJ] communities). The young and older adults generally have less resistance to infections. Children, especially malnourished children, may be more likely to exhibit severe effects of AGI after exposure to some pathogens (e.g., pathogenic *E. coli*, some enteric viruses). However, some pathogens (e.g., Hepatitis A, poliovirus) may cause less clinical illness in children than in adults (Gerba et al.,

1996). Age can also contribute to different exposure patterns due to behavior. For example, as discussed above, children have higher levels of incidental ingestion of water during swimming than adults (Dufour et al., 2017; DeFlorio-Barker et al., 2017).

Populations considered immunocompromised or immunosuppressed due to recent or concurrent illness or medical treatment may be defined as subpopulations that risk assessment could address (Effler et al., 2001). For clarity, definitions of subpopulations included in a risk assessment would include the criteria used to classify individuals as immunocompromised and could be limited to specific identifiable types of immune defects. For example, extreme physical or emotional stress can lower immune competency (Schneiderman et al., 2005; Dhabhar, 2014). The host gastrointestinal environment can vary in ways that affect pathogens, and innate immunity also plays a role in infection dynamics (Yoo et al., 2020). Malnourished individuals tend to have weaker immune defenses than well-nourished individuals (Schaible et al., 2007). Persons with concurrent illness or undergoing medical treatments may have increased susceptibility (Morris et al., 1997; Dropulic and Lederman, 2016). Genetic background can also affect immune status but may play a larger role in the mechanism of infection and disease progress (U.S. EPA, 2014b).

Previous exposure can confer limited, short-term, or longer-term protective immunity for some pathogens (Frost et al., 2005). The converse of this may also be true; when individuals or populations that have not previously been exposed to particular pathogens, infection and illness rates can be higher than would otherwise be anticipated. “Traveler’s diarrhea” is a well-known observed phenomenon that exemplifies this type of situation. Pregnancy may cause women to be more susceptible to a pathogen. For example, Hepatitis E, which causes a self-limiting disease in most infected persons, can cause up to 20% mortality in women in the third trimester of pregnancy (Jameel, 1999). Pregnancy can also affect behaviors, such as increased water consumption.

Social and cultural behavioral traits primarily affect exposure patterns. For example, a relatively small proportion of the population is responsible for consuming the majority of raw and partially cooked shellfish (FDA, 2005). Cultural traditions such as subsistence fishing can increase exposure to water. Location combined with cultural traditions, such as Asian and Pacific Islander (e.g., Hawaiians) engagement with the ocean, can influence exposure levels. Tribal exposures may not fall into primary or secondary contact categories, as lifestyle exposures go beyond both of those categories. Surfers also have lifestyles that have higher levels of exposure to recreational waters than is normally considered (Arnold et al., 2017).

EJ is the fair treatment and meaningful involvement of all people, regardless of race, color, national origin, or income, with respect to the development, implementation, and enforcement of environmental laws, regulations, and policies.[29] Executive Order 12898, Federal Actions to Address Environmental Justice in Minority Populations and Low-Income Populations, directs federal agencies to:

- Identify and address the disproportionately high and adverse human health or environmental effects of their actions on minority and low-income populations to the greatest extent practicable and permitted by law.
- Develop a strategy for implementing EJ.

[29] https://www.epa.gov/environmentaljustice

- Promote nondiscrimination in federal programs that affect human health and the environment and provide minority and low-income communities access to public information and public participation.

EJ communities may have higher proportions of risk factors due to limited access to medical care and affordable and nutritious food.

4.2 Sensitivity Analyses

A sensitivity analysis is the practice of changing a parameter value in a risk assessment to determine how big of an effect that parameter has on the results and to better understand the results of the base analysis. Three sensitivity analyses were performed to evaluate how choosing different parameters or parameters for different populations affected the QMRA results. The questions these analyses addressed were:

1. Considering the nonhuman fecal source, seagulls, how does more recently available information in the scientific literature affect previously published QMRA health modeling results (Soller et al., 2014)? The analysis in Soller et al. (2014) is compared to an updated QMRA analysis incorporating revised parameter choices based on available scientific literature and presented in this TSM (Section 4.2.1).

2. What effect does the choice of ingestion volume parameter have on QMRA results characterizing a mixture of human and nonhuman fecal contamination when the ingestion for the general population is compared to the ingestion volume for children 6 to 10 years old (Section 4.2.2)?

3. Does the inclusion of more recently available dose-response information for norovirus (Teunis et al., 2020) affect the viral etiology conclusions from Soller et al. (2010a) (Section 4.2.3)?

4.2.1 Compare Gull Analysis from Soller et al. (2014) to this TSM

Soller et al. (2014) reported the predicted risk for a mixture of human and nonhuman (gulls, pigs, or chickens) fecal contamination in varying proportions from 0%–100% human and predicted enterococci densities that correspond to the 36 NGI per 1,000 recreators target illness rate in the 2012 RWQC (these densities are referred to hereafter as RBTs). A recent literature search of relevant QMRA parameters identified multiple potential updates that were included and discussed in this TSM (Section 2.1). As part of that update, the EPA updated the analysis presented in Soller et al. (2014) to understand the effect of these changes on the QMRA modeling output. This sensitivity analysis compares the RBT enterococci values reported for gulls in Soller et al. (2014) to the results from the Gull Case Study presented in this TSM (Appendix G).

Table 4-1 outlines the parameters that differ between the Soller et al. (2014) analysis and the revised Gull Case Study. The analysis used a reverse QMRA approach following the process presented in Section 2.2.2.4.5. While the Soller et al. (2014) modeling was originally conducted in the Mathcad computational environment, the EPA previously converted the Mathcad code to Python to make an open-source code option available. The converted Python code corresponding to the Soller et al. (2014) analysis is in Appendix J. The Gull Case Study in this TSM was conducted using Python code, which is available in Appendix H. While Appendix G comprehensively documents the Gull Case Study, the principal differences between the Soller et al. (2014) and the Gull Case Study may be broadly summarized as:

Table 4-1. Parameters that differ between Soller et al. (2014) and the gull case study in this TSM.

Parameter	Soller et al. (2014)	This TSM (gull case study; Appendix G)
Ingestion volume	19 mL/event	Table 2-8
Density of norovirus in raw sewage (10^n gene copies/L)	Uniform distribution with Min 3.0 Max 6.0	Log normal distribution (Table 2-2)
Density of enterococci in gull feces (10^n CFU/L)	Min = 6 Max = 8	Table 2-7
Density of *E. coli* in gull feces (10^n CFU/L)	Min = 5 Max = 9	Table 2-7
Dose-response norovirus	$\alpha = 0.04$ $\beta = 0.055$	Table 2-5 Stochastic z and w intermediate parameters used to estimate distributions of α and β
Dose-response *Campylobacter*	$\alpha = 0.024$ $\beta = 0.011$	Table 2-5 Stochastic z and w intermediate parameters used to estimate distributions of α and β
Infection to illness norovirus	Point estimate 0.6 (60%)	Table 2-6 Stochastic z and w intermediate parameters used to estimate distribution of r and η
Infection to illness *Campylobacter*	$r = 2.44 \times 10^8$ $nu = 3.63 \times 10^{-9}$	Table 2-6 Stochastic z and w intermediate parameters used to estimate distribution of r and η

- Updates to the density of pathogen and indicator levels found in human and gull contamination, which were implemented by straightforward parameter updates to the 2014 analysis code.
- Updates to the dose-response functions used to describe the relationship between pathogen ingestion and risk of illness, which required more structural changes to the 2014 analysis code. Specifically, the new dose-response functions required the intermediate generation of stochastic estimates (statistical distributions) of the parameters of the dose-response functions, which were then used to estimate a distribution of risk at the modeled dose. The median of the risk distribution was thereafter used as a point estimate of the risk corresponding to the modeled dose.
- Recalculation of the relative contribution of raw human contamination and human effluent to the human source mix consistent with the new pathogen and indicator densities.

The results of the sensitivity analysis are presented in Table 4-2 and Figure 4-1. Note that the results are so similar that the icons mostly overlap on Figure 4-1. Given these results, it can be concluded that the updated parameters, including the updated norovirus dose-response function, did not have a meaningful impact on the results.

Table 4-2. Predicted median enterococci densities that correspond to illness levels of 36 NGI per 1,000 recreators (RBT) for waters impacted by mixed sea gull and human fecal contamination.

Nonhuman source	Percent human contribution				
	10%	20%	30%	50%	100%
Gull (Soller et al., 2014)	339	174	116	70	35
Gull (this TSM gull case study)	349	175	117	70	35

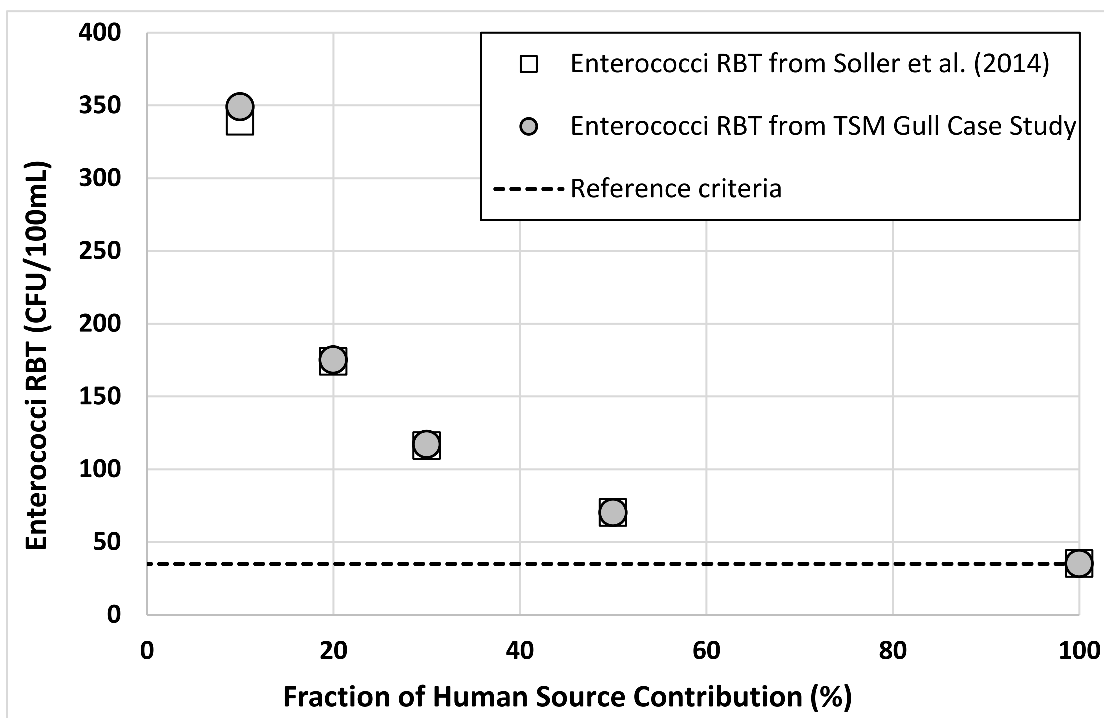


Figure 4-1. Enterococci RBTs for recreational water contaminated by gulls and humans, resulting in 36 NGI per 1,000 recreators. Dotted horizontal line is 35 enterococci CFU/100 mL (the RWQC magnitude).

4.2.2 Compare Children's Exposure to General Population

The EPA conducted a sensitivity analysis to compare the results of the reverse QMRA approach presented in this TSM depending on whether the incidental ingestion parameter was for the general population or children 6 to 10 years old (see Section 2.2.2.4.4 for the methodological description and Appendix G for the base analysis). The median incidental ingestion parameter for the general population is 19 mL per recreational event (base analysis) and 25 mL per recreational event for children aged 6 to 10 (sensitivity analysis). The analysis considered different fractions of contamination from human sources (10%, 25%, and 33%) in relation to the contribution of seagull fecal contamination.

This sensitivity analysis demonstrates that the ingestion parameter noticeably influences the calculated enterococci RBT. For each proportion of human fecal contamination in the mixture, the calculated enterococci levels corresponding to 36 NGI per 1,000 recreators were consistently lower for children than the general population. As the percentage of human contribution increases, the enterococci RBT decreases. Including the increased incidental ingestion for children 6 to 10 years old also corresponded to a lower RBT for enterococci, but the child-based RBT is still greater than the recommended value in the 2012 RWQC. In the scenario where gull contamination is mixed with human sources, the enterococci criteria levels would be 24% lower than the levels for the general population, regardless of the level of human contamination. This corresponds to 25 CFU lower at the 33% human contamination level and 84 CFU lower at the 10% human contamination level (Table 4-3). Results from a nonpathogenic source of enterococci are shown in Appendix G and are similar enough to the gull results that the icons would overlap on Figure 4-2.

Figure 4-2 shows the derived enterococci criteria levels on the Y-axis in CFU per 100 mL and the percentage of human fecal contribution on the X-axis. The figure shows six different contamination scenarios: 10%, 20%, 25%, 30%, 33%, and 50% contribution of human fecal contamination. The remaining nonhuman contribution of enterococci is from seagulls. This sensitivity analysis shows that hypothetical enterococci criteria levels for this illustrative scenario, derived through the approach described in this TSM, would be lower for children (squares) compared to the general population (circles).

Table 4-3. Enterococci RBTs (CFU/100 mL) at different fractions of human fecal contamination (%).

Nonhuman source of fecal contamination & ingestion volume	Susceptibility of waterbody to human fecal contamination (percent human contribution)				
	Low			Medium	High
	≤ 10%	≤ 25%	≤ 33%	34–67%	> 67%
Gulls (19 mL median ingested volume, gen. population)	349	140	106	**Nationally recommended RWQC apply**	
Gulls (25 mL median ingested volume, children)	265	106	81		

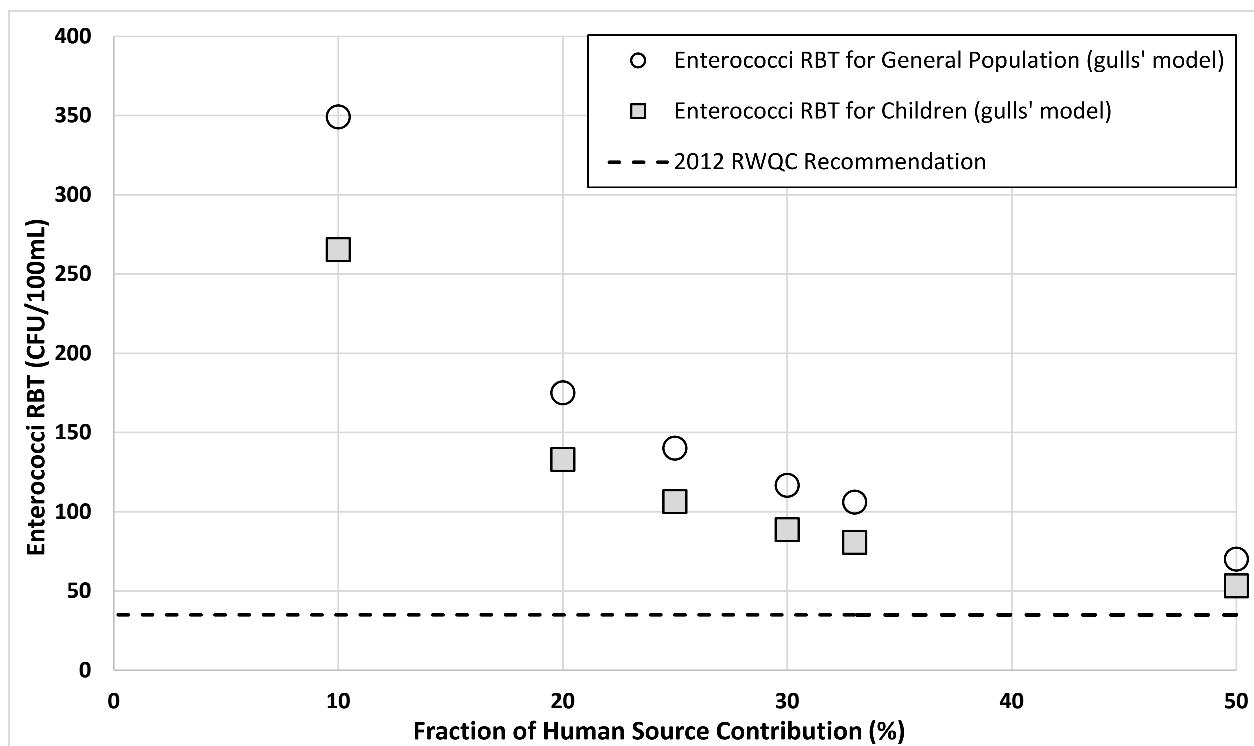


Figure 4-2. Enterococci criteria levels for recreational water contaminated by gull and human fecal mixture, resulting in 36 NGI per 1,000 recreators. Dotted horizontal line is 35 enterococci CFU/100 mL.

4.2.3 Confirmation of Viral Etiology for Human Fecal Sources

Soller et al. (2010a) used a QMRA-based approach to understand more fully the reported NEEAR results for the study sites on the Great Lakes. Recreational water epidemiological studies do not typically provide information about the specific pathogens, or etiologic agents, that can be responsible for the reported swimmer illnesses. Soller et al. (2010a) estimated the likelihood of illness from recreational exposure to enteric pathogens using a standard list of reference pathogens. They evaluated the time-to-onset-of-illness data to conclude that human enteric viruses, as indexed by the norovirus dose response, were likely responsible for the vast majority of the reported swimming-associated NGI at the NEEAR study sites. The norovirus dose-response function became a central parameter for the establishment of the etiologic agent of illness in the NEEAR study, conducted at sites primarily affected by secondary treated and disinfected effluent, and allows norovirus to be considered as an index pathogen when characterizing potential risks from exposure to human fecal sources (Section 2.1.1). The EPA revisited the 2010 analysis in 2022 to consider the effect of more recently available parameter information on the original conclusions.

The norovirus dose response in the 2010 analysis was reported by Teunis et al. (2008a) and used median point estimates for the α and β terms in the dose-response equation. Since 2010, there has been an update to the norovirus dose response that includes a meta-analysis of clinical challenge studies and norovirus outbreak data (Teunis et al., 2020). The Teunis 2020 dose-response relationship is a more complex function that uses a mean vector and covariance matrix to define a bivariate normal distribution of the infection dose-response parameters and another for the illness dose-response parameters. For the purposes of this sensitivity analysis and consistent with Soller et al. (2010a), a simplified version of that dose-response relationship was used based on the median parameters for

both infection (α, β) and illness (r, η). Specifically, the median values for infection from norovirus genogroup I (α, β) for secreter-positive individuals and the median values for illness (r, η) based on the outbreak-based observations were used (Tables 2-5 and 2-6, Section 2.1.1).

The EPA also included updated parameters for incidental ingestion and dose-response relationships for the reference pathogens adenovirus and *Campylobacter*. For adenovirus, the median values for infection (α, β) and illness (r, η) were based on the oral route of ingestion (Teunis et al., 2016; also evaluated three other routes of exposure). For *Campylobacter,* the median values for infection (α, β) and illness (r, η) were based on the values predicted by Teunis et al. (2018) based on a compilation of challenge study data (noting that challenge studies and outbreaks resulted in similar infection dose-response relationships; in outbreaks, the doses required to cause illness were lower than in challenge studies).

The original Mathcad code (not shown) used for Soller et al. (2010a) was modified as summarized in Table 4-4. In these sensitivity analyses, the EPA revisited the Soller et al. (2010a) analysis to evaluate a set of parameter changes on the model outputs to test that the main conclusions in Soller et al. (2010a) were still relevant. Specifically, the EPA evaluated changes to the ingestion, norovirus dose response, adenovirus dose response, and *Campylobacter* dose-response parameter selections (Table 4-4; parameters that were not changed are not shown in Table 4-4.)

The 2010 analyses were conducted using two different approaches to estimate pathogen densities consistent with reported illness rates. The first, a “health-based approach,” assumed that swimming-associated gastrointestinal illnesses occurred in the same proportion as projected illnesses from known pathogen occurrence in the United States. The second, a publicly owned treatment works (POTW) “POTW effluent-based approach,” assumed pathogens occurred in the recreational waters in the same proportion as in disinfected secondary effluent. In these sensitivity analyses, the same approach was maintained as was used in 2010 to facilitate comparisons and identify the extent to which parameter selection impacts the overall findings reported in 2010.

Table 4-4. Parameter comparison between Soller et al. (2010a) and the 2022 Etiology Analysis.

Parameters	Parameter detail	2010 (Soller et al., 2010a)	2022 Etiology Analysis
Ingestion	Mean general population	33 mL (Mean)	44 mL
Dose-response	Norovirus hypergeometric	α = 0.04	α = 0.393
		β = 0.055	β = 0.767
	Adenovirus exponential	0.4172	α = 5.11
			β = 2.8
	Campylobacter hypergeometric	0.024	α = 0.44
		0.011	β = 0.51
Probability of illness given infection	Norovirus	0.6 (60%)	r = 3.19
			η = 0.801
	Adenovirus	0.5 (50%)	r = 0.41
			η = 6.53
	Campylobacter	0.028 (2.8%)	r = 0.06
			η = 0.88

Using the reported health association and the daily measured water quality from the freshwater NEEAR study sites resulted in a mean illness rate of 30.6 illnesses per 1,000 recreators (Soller et al., 2010b). For the health-based approach, the results (in terms of illness rate per 1,000 swimmers) for these sensitivity analyses were the same as reported in 2010 because the ratio of illnesses was a fixed as part of the methodology. The results of these sensitivity analyses for the "POTW effluent-based" approach are shown in Table 4-5. The results from the 2010 analysis are presented first for comparison (Base Analysis). Changing the ingestion from 33 mL to 44 mL did not impact the results, as shown in the Sensitivity Analysis 1 column of Table 4-5. Updating the norovirus dose response based on the results reported by Teunis et al. (2020) had a minor influence on the quantitative results, where the fraction of illnesses attributed to norovirus in the analysis decreased from 29.7 to 29.3 NGI per 1,000 recreators (Sensitivity Analysis 2); in contrast, the fraction of illnesses attributed to other pathogens changed less than 0.1 NGI per 1,000 recreators. Updating all the parameters to the most recent values (Teunis et al., 2016, 2018, 2020, and updated ingestion values) yielded results very similar to the Base Analysis (Sensitivity Analysis 3).

Taken together, these sensitivity analyses support the original findings from the 2010 analyses that human enteric viruses, as indexed by the norovirus dose response, were likely responsible for the vast majority of the reported swimming-associated NGI at the NEEAR study sites, and that norovirus can be considered as an index pathogen when characterizing potential risks from exposure to human fecal sources.

Table 4-5. Illness rate per 1,000 swimmers for the POTW effluent-based approach.

	Base analysis	**Sensitivity analysis 1**	**Sensitivity analysis 2**	**Sensitivity analysis 3**
Pathogen	**2010 ingestion and dose response**	**2022 ingestion and 2010 dose response**	**2022 ingestion and 2022 norovirus dose response**	**All updated parameter values***
Rotavirus	0.3	0.3	0.4	0.4
Norovirus	29.7	29.7	29.3	29.8
Adenovirus	0.3	0.3	0.4	0.00015
Cryptosporidium spp.	0.2	0.2	0.3	0.34
Giardia lamblia	0.01	0.01	0.02	0.02
Campylobacter jejuni	0.1	0.1	0.2	0.003
E. coli O157:H7	0.001	0.001	0.001	0.001
Salmonella enterica	0.0003	0.0003	0.0004	0.0004

Note: * Includes updated dose response and probability of illness given infection for norovirus, adenovirus, and *Campylobacter* (as shown in Table 4-4).

4.3 Strengths and Uncertainties of Approach

The strengths and uncertainties of this TSM approach are largely based on how the user implements the approach. Throughout the TSM, users are provided with information to help them conduct each step with transparency and flexibility to improve parameters with location-specific data. When high-quality location-specific data are included, the approach provides the best fit for public protection at the location(s) characterized.

Site selection is an important aspect of the approach and can result in increased strength or, alternatively, greater uncertainty. Whether site selection is a strength or a limitation depends on how representative the selected sites are of the locations where potential alternative criteria developed using the process described in this TSM will apply. The representativeness of the sites can affect the applicability of the RBTs developed and decisions made using the TSM approach.

Because pathogen densities in fecally contaminated ambient waters can be highly variable and the associated monitoring results can include many nondetects, the representativeness of the monitoring approach included in the SAP is also important to consider. Users should have a plan to address nondetects in their analysis. A robust distribution of pathogen data above the detection limit will result in better-quality risk estimates in the QMRA. It is important to reiterate that the monitoring approach used to enumerate the reference pathogens reflects the expected hazardous condition, i.e., when pathogen loading to the waterbody being studied is expected to occur. If the sanitary survey information collected in Step 1 identifies that nonhuman fecal source loading to surface waters is expected following rain events, then the monitoring approach should reflect that pattern. If fecal loading is event-driven, monitoring base flow conditions may not reflect the hazardous condition. A transparent documentation of representativeness allows for a better assessment of the uncertainties. For example, if the user is characterizing beach sites affected by gulls, and the overall goal is to apply alternative criteria developed using this TSM broadly to gull-affected beaches in a state, the user would need to identify specific site characteristics and water quality data collection that support representativeness.

The approach presented in this TSM is designed to be a methodical stepwise process to inform transparent decision-making. As shown in Sections 1 and 2, the approach includes a sanitary characterization (sanitary survey and water quality study), evaluation of human health risks (forward QMRA), and derivation of alternative RBTs for culturable enterococci or *E. coli* (reverse QMRA). The approach in this TSM is to provide transparent documentation of each of step when deriving alternative WQC.

A strength of the sanitary characterization approach in this TSM is that it includes two types of information: a sanitary survey and a water quality study that are specific to the location being studied. Together, these sources of information can be used to identify fecal sources, confirm or dispel assumptions about fecal loading and contamination dynamics, and delineate the representativeness of the data collected. Identification of the fecal sources is an important outcome of the sanitary characterization. The better the characterization of fecal sources, the better the confidence in the outcome of the TSM approach.

There are inherent limitations of water quality methods used to enumerate microorganisms and with monitoring approaches that do not characterize matrix variability; these limitations apply broadly to microbial water quality assessment, and a specific discussion on these topics is beyond the scope of

this TSM. However, a discussion of the specific limitations of the water quality methods that are chosen for the water quality study is helpful for assessing the quality of the data. For example, the characterization of limits of detection, target specificity, number of replicates, and sample size are important for transparent interpretation and communication of water quality data.

4.3.1 QMRA

QMRA is a popular approach to aid in understanding the risks associated with exposure to recreational waters (see Table 1-2 in Section 1.2 for summaries of QMRA studies). QMRA has been used in several studies to examine the effects of recreational exposures to both human and animal fecal contamination on human health (Ashbolt et al., 2010; Schoen and Ashbolt, 2010; Soller et al., 2010a,b; U.S. EPA, 2010a; Schoen et al., 2011). It has also been highlighted as a tool that could be useful for developing alternative RWQC for sites impacted by nonhuman sources (U.S. EPA, 2007a,b; Boehm, 2009; Dorevitch et al., 2010; Soller et al., 2010a,b).

The QMRAs presented in this TSM have some features that are related to choices that define the scope of the scenarios that are covered by the QMRA. Scope-related features include:

- **Gastrointestinal illness as the health outcome of primary concern**: Based on epidemiological investigations, skin infection and disease, conjunctiva infection and disease, and ear infections and disease have not been typically shown to be associated with culturable FIB (Prüss, 1998; Wade et al., 2003; Zmirou et al., 2003). Although an association with culturable FIB has, in some cases, been shown with respiratory infection and illness (Fleisher et al., 1996) or skin illness (Sinigalliano et al., 2010), associations with gastrointestinal illness have been documented more frequently; therefore, gastrointestinal illness rates predicted by the QMRA or established in epidemiological studies are assumed to be protective for respiratory illness.

- **Selection of reference pathogens**: The reference pathogens that were selected all have illness endpoints that include gastrointestinal illness. Additional or other reference pathogens could be chosen to cover other illness endpoints.

- **Incidental ingestion during recreational activities is the exposure route of interest**: Other routes of exposure, such as inhalation and dermal contact, have not been shown to substantially add to the risk associated with ingestion.

- **The approach does not include secondary transmission in the health modeling**: Because criteria for recreational waters are recommended for recreators engaging in primary contact recreation, the health modeling does not include secondary transmission. In this context, illness is considered the result of event-based exposure to recreational waters containing fecal contamination. Secondary transmission is not considered within the scope of determining a health burden or target level of protection of criteria recommendations. In the QMRA framework discussed in this TSM, the scope of the scenario does not include person-to-person illnesses, nor does it explicitly address infectious disease transmission attributes (e.g., person-to-person transmission and immunity). Although person-to-person transmission and immunity can influence risk in unintuitive ways (Riley et al., 2003; Eisenberg et al., 2004, 2008; Soller et al., 2006; Soller, 2009), including these parameters does not substantially affect the estimated risks for the recreational exposure scenarios considered in this TSM (Soller and Eisenberg, 2008).

Assumptions underlying the QMRA include the following:

- Loss of pathogen virulence due to passage through nonhuman hosts or exposure to a nonenteric environment can be characterized as “high,” “medium,” and “low” in the QMRA based on the relative occurrence of species that infect humans and strains and serotypes present in typical livestock wastes.
- Human dose-response models adequately predict infection or illness risks for reference pathogens, regardless of the source (though variability in host-pathogen system response can be included in dose-response modeling).

4.3.1.1 Strengths

Applying QMRA to evaluate recreational waters has developed over the last several decades and has established a strong scientific foundation in the peer-reviewed literature. The general QMRA approach is well-accepted and grounded in peer-reviewed approaches and practices that are thoroughly well-documented. Numerous QMRA-based studies have been peer reviewed and published in scientific journals, and a standard of practice is now well established.

The QMRA methodology discussed in this TSM is sufficiently flexible for evaluating a range of source inputs and exposure scenarios. Published QMRA examples in the literature have characterized human health risks from exposure to many media types, including drinking water, recreational water, biosolids, and potable and nonpotable recycled water. Data are also available to address recreational exposure differences among lifestages.

The QMRA process described in this TSM is transparent in the assumptions made and input parameters chosen. QMRA can also be used to (1) evaluate sensitivity and uncertainty analyses, which can help test parameter assumptions and choices, and (2) identify potential data gaps, which can inform research planning. The Problem Formulation in this TSM (Section 2.2) describes the parameter choices and the rationale for those choices. Further, as new data and information become available, they can be straightforwardly incorporated into subsequent QMRA studies and the existing QMRAs can be efficiently revisited and revised if needed. Before new published parameter information is incorporated, it is recommended that users of this TSM evaluate the need to modify the existing parameters and the effect of the new information, such as with a sensitivity analysis. As with the discussion of incorporating newer dose-response information for norovirus (Section 4.2.1), the added complexity with the newer dose response did not substantially alter the results, so users can evaluate the need and potential tradeoffs when substantiating parameter selection in the risk assessment.

QMRA can directly address the etiological cause of illness, which is only indirectly addressed in epidemiological studies. In this TSM, a list of reference pathogens is recommended to estimate risk because these pathogens account for more than 97% of nonfoodborne illnesses in the United States (Mead et al., 1999; Scallan et al., 2011a,b). In contrast, recreational epidemiological studies most often characterize water quality in terms of a nonspecific surrogate for fecal contamination, such as culturable fecal indicator organisms. Additional reference pathogens could be added if desired.

Finally, an important strength of QMRA is the ability to evaluate situations that are difficult or impossible to evaluate otherwise. As discussed in Section 1, a strength of QMRA is its ability to add additional insight for risk management in waters not represented by traditional epidemiological-based

approaches or in waters where it may be impractical to conduct epidemiological studies (i.e., too few swimmers). In addition, in some cases, epidemiological approaches fail to establish predictive relationships between the FIB and reported illnesses. A strength of QMRA is that it does not have these potential limitations of epidemiological studies and can be cheaper to conduct than epidemiological studies.

4.3.1.2 Uncertainties/limitations (data availability)

The QMRA approaches presented in this TSM have associated limitations and uncertainties. These limitations and uncertainties fall into two main categories: (1) methodological and (2) data limitations. In some cases, the EPA has attempted to address these limitations in the ways discussed below.

Methodological:

The QMRA framework discussed in this TSM includes eight reference pathogens that are surrogates for the three major groups of pathogens and account for more than 97% of nonfoodborne illnesses in the United States (Mead et al., 1999; Scallan et al., 2011a,b). The TSM approach is flexible and can include additional pathogens in the framework. A limitation of the reference pathogen approach is that it does not include all pathogens. Although many pathogens are known, not all are known, and new ones can emerge over time. It is also the nature of a QMRA to generate a predicted level of illness rather than describe a reported case symptomology as in the case of an epidemiological study. The EPA has attempted to address this limitation by characterizing, or "anchoring," the performance of the health models using the assumptions and information listed above against the empirical outcomes reported in recreational water epidemiological studies. For example, in Soller et al. (2010b), incubation time and illness duration in swimmers and nonswimmers in the NEEAR epidemiological study were used to evaluate the plausibility of the QMRA results. The time-to-onset of illness data for nonswimmers and the predicted pathogens for the epidemiology studies were used to estimate the distribution of the time-to-onset of gastrointestinal illness in swimmers. That distribution was then compared to the time-to-onset of gastrointestinal illness in swimmers reported during the water epidemiology studies to evaluate the feasibility of the predictions about the pathogens present during the water epidemiology studies described in the previous sections. The results of the estimated time-to-onset of illness in swimmers were very similar to the observed time-to-onset of illness in swimmers who participated in the NEEAR study.

In other examples, QMRA was conducted in concert with epidemiological studies to provide additional information and interpretation of the results (Soller et al., 2016; Soller et al., 2017). Soller et al. (2016) describe a QMRA conducted using pathogen data collected during the EPA's epidemiological study in Boquerón, Puerto Rico. The QMRA calculated a mean swimming-associated illness level of approximately 2 NGI per 1,000 recreators, a rate of illness below the statistical power the epidemiological study was designed to detect (i.e., 17 NGI per 1,000 recreators). Soller et al. (2017) incorporated site-specific pathogen monitoring data of stormwater collected during an epidemiological study characterizing risks from wet weather impacts in an urbanized watershed. Results were consistent with enteric viruses, such as norovirus, as an important cause of gastrointestinal illness among recreators. Coupling QMRA with an epidemiological study at a single site provides additional utility for understanding illness and informing decisions.

Data Limitations:

Data availability and data representativeness are important concerns for selecting parameter values. A wide range of data quality is associated with potential datasets that could be used in QMRA studies.

- Variations Among Pathogen Strains: Accounting for strain heterogeneity in pathogens is challenging because dose-response data are not available for all strains, and not all pathogen strains have been identified or tested. The simplest means to predict response for exposure to a variety of strains is to ignore inter-strain variations by using a dose-response model based on pooled data, using a dose-response model based on the most virulent strain among the strains considered, or selecting a dose-response model for a "representative strain." In some cases, models of pooled data might not exhibit goodness of fit (Coleman and Marks, 1998). A more systematic technique for addressing strain-to-strain variation is described by Soller et al. (2007). In that study and drawing from the previous work of Coleman and Marks (1998, 2000), Gompertz-log dose-response models (alternatively called the Weibull dose-response model) were fitted to data for all strains of *Salmonella* for which data were available.

- Accounting for Differential Susceptibility in Dose Response: Intrinsic and extrinsic factors modify the effect of a specific exposure on the risk or severity of outcomes in an individual population (Balbus et al., 2000). Intrinsic factors include age, gender, prior disease, immune status, pregnancy, and diabetes, and extrinsic factors include residence, income, co-exposures, access to health care, and behaviors (Lanciers et al., 1999; Currie et al., 2000; Parkin et al., 2003; Makri et al., 2004; Parkin, 2004; WHO, 2005; Dietert et al., 2010). Dose-response is related to the intrinsic factors that affect how likely a person is to become infected or ill after exposure to a pathogen.

- Fate and Transport of Microbes in Environmental Waters: Hydrodynamic and transport models have been used to characterize changes in pathogen densities for various scenarios, including watershed scale (a review of watershed transport models is found in Coffey et al. [2007]); coastal waters (e.g., by Liu et al., 2006); for mixing in a river reach downstream of WWTP discharge (Soller et al., 2003); CSO-impacted river and bay (King County Department of Natural Resources, 1999); in an impaired waterbody (Soller et al., 2006); and for fate and transport of manure-based pathogens (Martinez et al., 2014).

4.4 Future Directions/Research Needs

The scope of this TSM is based on the scope of the 2012 RWQC. This discussion is limited to potential future information relevant to the current scope of the TSM. Therefore, directions that would expand the scope of the TSM are not included here.

Additional information that could be informative at the local scale includes:

- Fate and transport of pathogens and indicators: improved understanding of specific scenarios to provide information for IEM.

- Quantitative source characterization: improved methods for identifying and quantifying fecal sources, including more data collected at locations of interest.

- Pathogen profiles: more characterization of pathogen profiles from fecal sources across geographic regions.
- Exposure scenarios: expand understanding of different exposure scenarios.

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Appendix A: Literature Search Strategies

This appendix provides information on how literature was found for this TSM. The EPA developed a set of literature search strategies based on the Critical Path Science Plan that were previously published.[30] The peer-reviewed scientific literature was searched for recreational exposure information and relevant data for reference pathogens and fecal indicators. The resulting literature from the following published literature search strategies were examined for relevance for this TSM. These literature search strategies can be found in:

- Report on the 2nd Five-Year Review of EPA's Recreational Water Quality Criteria (U.S. EPA, 2023).
- 2017 Five-Year Review of the 2012 Recreational Water Quality Criteria (U.S. EPA, 2018). (Includes a targeted literature search for relevant QMRAs.)
- Appendix A of Review of Published Studies to Characterize Relative Risks from Different Sources of Fecal Contamination in Recreational Water (U.S. EPA, 2009a).
- Appendix B of Review of Zoonotic Pathogens in Ambient Waters (U.S. EPA, 2009b).
- Literature Search Strategy in State-of-the-Science Review of Quantitative Microbial Risk Assessment: Estimating Risk of Illness in Recreational Waters which is Annex 1 of Quantitative Microbial Risk Assessment to Estimate Illness in Freshwater Impacted by Agricultural Animal Sources of Fecal Contamination (U.S. EPA, 2010a).
- Appendix B of Assessment of the Extra-Enteric Behavior of Fecal Indicator Organisms in Ambient Waters (U.S. EPA, 2010b).

Additionally, citation mining (reverse searches) was conducted for the following publications:

- Microbiological Risk Assessment (MRA) Tools, Methods, and Approaches for Water Media (U.S. EPA, 2014b).
- Recreational Water Quality Criteria (U.S. EPA, 2012).
- Recommended Human Health Recreational Ambient Water Quality Criteria or Swimming Advisories for Microcystins and Cylindrospermopsin (U.S. EPA, 2019).
- Guidelines on Recreational Water Quality. Volume 1: Coastal and Fresh Waters (WHO, 2021).
- Soller, J.A., M.E. Schoen, T. Bartrand, J.E. Ravenscroft, and N.J. Ashbolt. 2010b. Estimated human health risks from exposure to recreational waters impacted by human and nonhuman sources of faecal contamination. *Water Research* 44(16):4674–4691. (Includes primary citations for parameters related to pathogens and FIB in feces and dose-response models.)

[30] https://www.epa.gov/wqc/research-supporting-development-2012-recreational-water-quality-criteria

Subject matter experts, authors, and reviewers listed in the acknowledgements page suggested additional references to include.

Targeted literature searches were conducted from 2011 to 2021 during the development of the TSM to supplement the literature searches listed above. The most recent literature search to update parameters is shown in Table A-1. Of the 122 title/abstracts returned by the literature search, full text was reviewed for 37, and 12 of those had new information on parameters. Some of the 12 had duplicate information, so only adenovirus and norovirus had new information that was incorporated into the latest iteration of the modeling.

A targeted literature search was conducted for gull feces parameters. The pathogen prevalence, density of pathogens, and fraction that are human infectious, were the parameters of interest. The following searches were conducted:

- Google Scholar on 5/2/2022 for terms “enterococci and gull and feces and culture” with restrictions of 2011–2022.

- Google Scholar on 5/4/2022 for terms “gull and feces and Campylobacter” with restriction to 2011–2022.

The searches resulted in 1,800 titles, sorted by relevance. The top 30 titles were screened from each search. Six articles were reviewed in full text. The cited articles from two reviews, Minias (2020) and Smith et al. (2020), were used to identify additional articles that contained prevalence and density data for *E. coli, Campylobacter*, and *Salmonella* in gull feces. In addition, 39 articles were reviewed that cited the most relevant EPA article, Lu et al. (2011).

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Table A-1. 2021 literature search for selected parameters.

Volume of water ingested during a primary contact recreation event			
	Search date	**PubMed[31] search string**	**Number of records identified**
	12/3/2021	("recreational water ingestion" OR "incidental ingestion of water" OR "oral exposure to water" OR "oral exposure recreational activities") AND ("volume of water") AND ("2017/01/01"[Date - Publication] : "2022/04/13"[Date - Publication])	6
Dose-response relationships and parameters for dose-response models for each reference pathogen			
Pathogen	**Search date**	**PubMed search string**	**Number of records identified**
Rotavirus	12/3/2021	("rotavirus") AND ("dose response" OR "dose-response") AND ("water") AND ("2017/01/01"[Date - Publication] : "2022/04/13"[Date - Publication])	3
Norovirus	12/3/2021	("norovirus") AND ("dose response" OR "dose-response") AND ("water") AND ("2017/01/01"[Date - Publication] : "2022/04/13"[Date - Publication])	16
Adenovirus	12/3/2021	("adenovirus") AND ("dose response" OR "dose-response") AND ("water") AND ("2017/01/01"[Date - Publication] : "2022/04/13"[Date - Publication])	6
Cryptosporidium	12/3/2021	("cryptosporidium") AND ("dose response" OR "dose-response") AND ("water") AND ("2016/01/01"[Date - Publication] : "2022/04/13"[Date - Publication])	9
Giardia lamblia	12/3/2021	("giardia" OR "giardia lamblia") AND ("dose response" OR "dose-response") AND ("water") AND ("2017/01/01"[Date - Publication] : "2022/04/13"[Date - Publication])	6
Campylobacter	12/3/2021	("campylobacter") AND ("dose response" OR "dose-response") AND ("water") AND ("2017/01/01"[Date - Publication] : "2022/04/13"[Date - Publication])	6
E. coli O157:H7	12/3/2021	("E. coli O157:H7" OR "Escherichia coli O157:H7") AND ("dose response" OR "dose-response") AND ("water") AND ("2017/01/01"[Date - Publication] : "2022/04/13"[Date - Publication])	2
Salmonella enterica	12/3/2021	("salmonella enterica") AND ("dose response" OR "dose-response") AND ("water") AND ("2017/01/01"[Date - Publication] : "2022/04/13"[Date - Publication])	4

[31] https://pubmed.ncbi.nlm.nih.gov

Probability of illness given infection for each reference pathogen			
Pathogen	**Search date**	**PubMed search string**	**Number of records identified**
Rotavirus	12/5/2021	("rotavirus") AND ("probability of infection" OR "probability of illness" OR "morbidity") AND ("water") AND ("2017/01/01"[Date - Publication] : "2022/04/13"[Date - Publication])	22
Norovirus	12/5/2021	("norovirus") AND ("probability of infection" OR "probability of illness" OR "morbidity") AND ("water") AND ("2017/01/01"[Date - Publication] : "2022/04/13"[Date - Publication])	16
Adenovirus	12/5/2021	("adenovirus") AND ("probability of infection" OR "probability of illness" OR "morbidity") AND ("water") AND ("2017/01/01"[Date - Publication] : "2022/04/13"[Date - Publication])	7
Cryptosporidium	12/5/2021	("cryptosporidium") AND ("probability of infection" OR "probability of illness" OR "morbidity") AND ("water") AND ("2017/01/01"[Date - Publication] : "2022/04/13"[Date - Publication])	27
Giardia lamblia	12/5/2021	("giardia" OR "giardia lamblia") AND ("probability of infection" OR "probability of illness" OR "morbidity") AND ("water") AND ("2017/01/01"[Date - Publication] : "2022/04/13"[Date - Publication])	21
Campylobacter	12/5/2021	("campylobacter") AND ("probability of infection" OR "probability of illness" OR "morbidity") AND ("water") AND ("2017/01/01"[Date - Publication] : "2022/04/13"[Date - Publication])	15
E. coli O157:H7	12/5/2021	("E. coli O157:H7" OR "Escherichia coli O157:H7") AND ("probability of infection" OR "probability of illness" OR "morbidity") AND ("water") AND ("2017/01/01"[Date - Publication] : "2022/04/13"[Date - Publication])	3
Salmonella enterica	12/5/2021	("salmonella enterica") AND ("probability of infection" OR "probability of illness" OR "morbidity") AND ("water") AND ("2017/01/01"[Date - Publication] : "2022/04/13"[Date - Publication])	14

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Appendix B: QMRA Sanitary Survey Form

The purpose of this Pollutant Source Characterization form is to provide a convenient and consistent mechanism to collect information about microbiological contamination from fecal sources for (1) short-term QMRA or epidemiological applications (e.g., site characterization) or (2) longer-term applications (e.g., AWQC site classification or site-specific AWQC).

The form is comprised of an overview form that is to be used to summarize high-level information about potential pollutant sources, followed by a watershed information form and a series of worksheets that are used to summarize details related to specific potential sources of fecal contamination in a watershed or at a recreational site. Justification for the data requested in specific sections is provided following the worksheets at the end of the document.

Logistically, it is expected that the watershed information form and the series of worksheets will be completed first. Then, that information will be used to provide the high-level summary information on the overview form.

The EPA has published a sanitary survey app, which can be accessed at: https://www.epa.gov/beach-tech/sanitary-surveys-recreational-waters.

SUMMARY OF POTENTIAL POLLUTANT SOURCES

Overall Evaluation of Pollutant Sources in Waterbody (narrative describing predominant source of contamination and justification)

Class and type of source	Supporting worksheet cross reference	Distance from site of recreational waterbody (feet)	Travel time to recreational waterbody (base / peak flow)	Relative contribution of contamination (H, M, L, NA) (base / peak flow)	Summary justification (Use numbers to cross reference with description in overall evaluation above)
HUMAN SOURCES Wastewater discharges	A, B, C				Type of treatment, disinfection, etc.
Sewage overflows and combined sewer overflows	A, B, C				Frequency, magnitude, duration
Septic systems and Unsewered areas	A, B, C				Number, proximity
Subsurface sewage disposal	A, B, C				Proximity, volume
Marinas, harbors, mooring boats	A, B, C				Proximity, number, temporal trends
Bathhouses	A, B, C				Number, proximity, potential for leakage
Designated swimming areas, beaches	A, B, C				Number of recreators, temporal trends

Class and type of source	Supporting worksheet cross reference	Distance from site of recreational waterbody (feet)	Travel time to recreational waterbody (base / peak flow)	Relative contribution of contamination (H, M, L, NA) (base / peak flow)	Summary justification (Use numbers to cross reference with description in overall evaluation above)
NONHUMAN INDIRECT SOURCES Stormwater outfalls	A, B, D				Number, volume, temporal trends
Urban runoff, industrial waste	A, B, D				Number, volume, temporal trends
Other drains and pipes	A, B, D				Number, volume, temporal trends
Wetland drainage	A, B, D				Stream volume, temporal trends
Groundwater seepage	A, B, D				Volume, relative contribution
Streams or natural outfalls	A, B, D				Volume, relative contribution
ANIMAL SOURCES CAFOs or AFOs	A, B, E				Number of animals, which animals
Other agriculture runoff	A, B, E				Number, which animals
Wildlife	A, B, E				Number, which animals
Domestic animals	A, B, E				Number, which animals
Birds	A, B, E				Number, temporal patterns
OTHER (specify)					

1.A. WATERSHED INFORMATION

Name of Watershed or Beach:	Date(s) of Survey:
Watershed ID:	Number of Routine Surveys Used:
Town/City/County/State:	Name(s) of Surveyor(s):
Sampling Station(s)/ID:	Surveyor Affiliation:
STORET Organizational ID:	

1.B INFORMATION ON WATER QUALITY SAMPLING LOCATION

Description of Sample Points

Sample point name/ID	Location	Description	Sample frequency	Time of day of sample collection

Description of hydrometric network (note that this is a network of monitoring stations that collect data such as rainfall and stream flow)

Comments/Observations:

WORKSHEET A
DESCRIPTION OF LAND USE, MAPS, AND PHYSICAL CONDITIONS

A.1 CURRENT LAND USE IN WATERSHED OR BEACH

Type	Residential	Industrial	Commercial	Agricultural	Other (specify):
Percentage					

Overall Development	Describe
% undeveloped	
% developed	

Waterbody Uses: ☐ Boating ☐ Fishing ☐ Surfing ☐ Windsurfing ☐ Diving ☐ Swimming

Are maps of the watershed attached? ☐ yes ☐ no ☐ Other (Specify)

List maps and their sources:

Does the detailed map include locations of:

Sample Points	☐ yes	☐ no	(explain):
Hydrometric Network	☐ yes	☐ no	(explain):
Pollutant Sources	☐ yes	☐ no	(explain):
Boat Traffic	☐ yes	☐ no	(explain):
Marinas	☐ yes	☐ no	(explain):
Boat Dockage	☐ yes	☐ no	(explain):
Fishing	☐ yes	☐ no	(explain):
Bathing / Swimming	☐ yes	☐ no	(explain):
Bounding Structures	☐ yes	☐ no	(specify: do they impact circulation?):
Residential Areas	☐ yes	☐ no	(explain):
WWTP Outfalls	☐ yes	☐ no	(explain):
Stormwater Outfalls	☐ yes	☐ no	(explain):
Other Drains	☐ yes	☐ no	(explain):
Sanitary Facilities	☐ yes	☐ no	(explain):
Septic Systems or Unsewered Areas	☐ yes	☐ no	(explain):
CAFOs or AFOs	☐ yes	☐ no	(explain):
Wetlands	☐ yes	☐ no	(explain):
Other	☐ yes	☐ no	(explain):

Photographs Taken in the Watershed or Beach Area

Image Number	Date/Time	File Name	Description of Photograph (Include Pictures of High Watermark Locations and Corresponding Fixed Objects)

A.2. PHYSICAL CONDITIONS OF WATERSHED OR BEACH

Recreational waterbody length or dimensions (indicate Z1, Z2, and Z3 on a map)

Length (m):	Width average (m):	Depth min, average, max (m):
Width Z1 (m):	Width Z2 (m):	Width Z3 (m):

Local water level variation: feet inches | Hydrographic influences:

Characterize any longshore or nearshore currents and their potential effects based on bacteria sampling results

Approximate beach slope at swim area (If appropriate): ____________ %

Summary description of recreational waterbody

Other Comments/Observations:

Outfalls	Number	Description or Comment
Streams or natural outfalls		
Storm drains		
WWTPs		
Other (specify):		
Other (specify):		

Beach Materials/Sediments:

☐ Sandy ☐ Mucky ☐ Rocky ☐ Other:

Is sand or sediment known to contribute to bacterial concentrations? (explain)

Habitat around beach: (Provide distance from recreational waterbody)

☐ Dunes ☐ Wetlands ☐ River/Stream ☐ Forest ☐ Park ☐ Protected Habitat or Reserve

☐ Other:

WORKSHEET B
WEATHER CONDITIONS, WATER QUALITY SAMPLING, AND MODELING

B.1. WEATHER

Examine the weather data collected over the prior recreational water season(s) along with bacteria sampling results.

Do the fecal indicator concentrations at this watershed or beach area appear to correlate with any of the following?

Rainfall	☐ yes	☐ no	(explain):
Air Temperature	☐ yes	☐ no	(explain):
Water Temperature	☐ yes	☐ no	(explain):
Cloud Cover	☐ yes	☐ no	(explain):
Wind Speed	☐ yes	☐ no	(explain):
Wind Direction	☐ yes	☐ no	(explain):
Other Weather	☐ yes	☐ no	(explain):

Have any statistical analyses been done to calculate the degree of correlation? ☐ yes ☐ no

Describe any analyses done, and any trends or correlations found (add lines if needed to describe in detail):

Rainfall total for the beach season (in):	Average rainfall for all beach seasons (in):
Number of significant rain events:	What constitutes "significant?" (e.g., 1 inch or more rain)

Additional Comments/Observations:

B.2. WATER QUALITY SAMPLING

Name of laboratory: ______________ Distance to laboratory: ______________ miles

Is there a sampling and analysis plan? ☐ yes ☐ no

Is it adequate? ☐ yes ☐ no (explain) ______________

Are the sampling staff properly trained on sampling techniques, equipment maintenance, and calibration procedures? ☐ yes ☐ no

Microbiological Sampling

Do you test for *Escherichia coli*? ☐ yes ☐ no Analytical Method Used: ______________

Do you test for *Enterococcus*? ☐ yes ☐ no Analytical Method Used: ______________

Do you test for fecal coliform? ☐ yes ☐ no Analytical Method Used: ______________

List any additional indicators or pathogens tested and associated analytical methods:

How do this past season's bacteria results compare to that of previous years? (explain if possible)

Do the indicator or pathogen results correlate to other parameters, such as water quality, weather, flow, bather load, algae, or wildlife?

(If yes, explain) ☐ yes ☐ no

Describe in detail analyses that were performed on historical data or that typically are performed on data that are collected.

Were there any unusual results, such as extremely high or low values detected, or unusual trends? ☐ yes ☐ no
If yes, explain what was found and any potential causes:

Are water quality annual trend data attached? ☐ yes ☐ no

Comments/Observations:

B.3. ADVISORIES/CLOSINGS

List any advisories and closings that occurred, whether bacteria levels were high, and any possible reasons for advisory or closing or high bacteria level, such as stormwater runoff, sewage spill, or wildlife on the beach.

Advisory or closing (specify one)	Start and end dates	Length of advisory or closing (days)	Did bacteria concentrations exceed GM or SSM criteria?	Reason for advisory or closing or possible contributing factors

Total number of closings issued: ____________ Total number of days under an advisory: ____________
Total number of advisories issued: ____________ Total number of days beach was closed: ____________

Comments/Observations:

B.4. MODELING

Are models being used to predict bacteria concentrations or the exceedance of bacteria standards? ☐ yes ☐ no
If yes, list types of models being used and a brief description of the models: ____________

Comments/Observations:

WORKSHEET C
Human Sources of Pollution

C.1. WASTEWATER DISCHARGE

Do the presence of wastewater discharges contribute to the fecal indicator concentrations at this watershed or beach area?

☐ yes ☐ no

If yes, please answer the following:

Facility name: ____________________

Does the facility discharge directly to the recreational water? ☐ yes ☐ no

Proximity to recreational water: (approximate distance in feet) ☐ < 500 ☐ 500–2,500 ☐ > 2,500

Discharge rate: ____________________

Approximate travel time to waterbody: ____________________

Is there a history of unintended discharge events? ☐ yes ☐ no

If yes, explain: ____________________

Describe treatment and disinfection employed at the treatment facility (Include temporal variability in treatment efficacy, if appropriate)

Do wet weather events affect the wastewater discharge? (e.g., additional flows, observed impacts to the recreational water)

If yes, describe:

C.2. SEWAGE OVERFLOWS

Does all of the watershed have municipal sewer? ☐ yes ☐ no

Are there sanitary sewer overflow pipes within the watershed?

☐ yes ☐ no ☐ unknown

If yes, describe location relative to recreational water: ____________________

Do the presence of sewage overflows contribute to the fecal indicator concentrations at this watershed or beach area?

☐ yes ☐ no

If yes, please answer the following:

Frequency of overflows: ____________________

Magnitude of overflows: (e.g., overflow volume) ____________________

Duration of overflows: ____________________

Are there specific conditions under which sewage overflows increase?: (e.g., rainfall events) ____________________

C.3. COMBINED SEWER OVERFLOWS

Are there combined sewer overflow pipes and/or drains within the recreational watershed?

☐ yes ☐ no ☐ unknown

Do the presence of combined sewer overflows (CSOs) contribute to the fecal indicator concentrations at this watershed or beach area?

☐ yes ☐ no

If yes, please answer the following:

Frequency of CSOs: ____________________

Magnitude of CSOs: (e.g., overflow volume) ____________________

Duration of CSOs: ____________________

Are there specific conditions under which CSOs increase?: (e.g., rainfall events) ____________________

____

Under conditions when CSOs occur, describe treatment and/or disinfection that occurs, if any:

____

C.4. SEPTIC SYSTEM AND UNSEWERED AREAS

Do the presence of septic systems or unsewered areas contribute to the fecal indicator concentrations at this watershed or beach area?

Septic systems: ☐ yes ☐ no Unsewered areas: ☐ yes ☐ no

If yes, please answer the following:

Proximity of septic system or unsewered area to recreational water: (approximate distance in feet) ____________________

Estimated volume of septic system or unsewered area: ____________________

Estimated area of septic system or unsewered area: ____________________

C.5. SUBSURFACE SEWAGE DISPOSAL

Is there subsurface sewage disposal at this watershed or beach area? ☐ yes ☐ no

If yes, please answer the following:

Proximity of subsurface sewage disposal to recreational water: (approximate distance in feet) ____________________

Estimated volume of subsurface sewage disposal: ____________________

C.6. MARINAS AND MOORING BOATS

Is there a marina or other boat moorings at this watershed or beach area? ☐ yes ☐ no

Boats moored within watershed: ☐ contain bilge tanks ☐ do not contain bilge tanks

Number of moorings: ____________________

Approximate number of boats present: (daily average) ____________________

Proximity of marina or moorings to the recreational water: (approximate distance in feet) ____________________

Does the presence of boats vary throughout the year (explain)?: (e.g., are there seasonal trends?) ____________________

____

C.7 DESCRIPTION OF BATHHOUSES OR SANITARY FACILITIES

Bathhouses: Total number of bathhouses/toilet facilities? Relevant to watershed or beach:

Number or ID	Location description	Condition (good, fair, poor)	Distance from beach (feet)	Frequency of cleaning (daily, weekly, monthly)

Describe further. Include number of toilets, showers, sinks, etc., and whether these facilities are adequate to support beach use.

C.8 BATHER LOAD (# OF BEACH USERS)

Is the number of bathers or recreators monitored? ☐ yes ☐ no

If yes, describe how beachgoer numbers are calculated (i.e., turnstile, counting at noon, photographs): ________________

Beach Use

Beachgoer category	Number of People Per Day Using the Beach					
	Peak use for the season (daily use)	Seasonal average (daily use)	Holiday average (daily use)	Weekend average (daily use)	Weekday average (daily use)	Off-season average, if applicable (daily use)
Total beach users						
People in the water						
People on beach but not swimming						
People boating						
People fishing						
People surfing						
People windsurfing						
People diving						
Other (specify):						
Frequency of measurements (e.g., biweekly, weekly, monthly)						

Examine bather density data along with microbial sampling results for the past beach season(s). Look at each sampling point. Does bather density appear to correlate with bacteria concentrations at any of these sampling points? Has a statistical analysis been done? Describe:

Comments/Observations:

WORKSHEET D
STORMWATER, URBAN RUNOFF, DRAINS, STREAMS, WETLANDS

D.1. STORMWATER

Are there stormwater overflow pipes and drains within the watershed?

☐ yes ☐ no ☐ unknown

Do the presence of stormwater runoff contribute to the fecal indicator concentrations at this watershed or beach area?

☐ yes ☐ no

If yes, please answer the following:

Type of impact: ☐ direct discharge ☐ indirect nonpoint source ☐ both direct and nonpoint sources

Proximity of discharge to recreational water: (approximate distance in feet) ______________________

Frequency of discharge: ______________________

Magnitude of discharge: (e.g., overflow volume) ______________________

Duration of discharge: ______________________

Is there a known or suspected predominant source of fecal contamination in the stormwater?

☐ yes ☐ no ☐ unknown

If yes, explain: ______________________

D.2. URBAN RUNOFF (NONSTORMWATER)

Does nonstormwater, urban runoff contribute to the fecal indicator concentrations at this watershed or beach area?

☐ yes ☐ no

If yes, please answer the following:

Are there any best management practices in place to control sources of urban runoff? ☐ yes ☐ no

Proximity of discharge to recreational water: (approximate distance in feet) ______________________

Frequency of discharge: ______________________

Magnitude of discharge: (e.g., overflow volume) ______________________

Duration of discharge: ______________________

Is there a known or suspected predominant source of fecal contamination in the urban runoff?

☐ yes ☐ no ☐ unknown

If yes, explain: ______________________

D.3. OTHER DRAINS AND PIPES

Are there other drains and pipes at this watershed or beach area?

☐ yes ☐ no

If yes, please answer the following:

Identify the type of drain or pipe: ______________________

Proximity of discharge to recreational water: (approximate distance in feet) ______________________

Frequency of discharge: ______________________

Magnitude of discharge: (e.g., overflow volume) ______________________

Duration of discharge: ______________________

Is there a known or suspected predominant source of fecal contamination in the drains or pipes?

☐ yes ☐ no ☐ unknown

If yes, explain: ______________________

D.4. WETLAND DRAINAGE

Is there a wetland drainage at this watershed or beach area?

☐ yes ☐ no

If yes, please answer the following:

Proximity of wetland drainage to the recreational water: (approximate distance in feet) ____________________

Frequency of drainage: ____________________

Magnitude of drainage: (i.e., drainage volume) ____________________

Duration of drainage: ____________________

Describe the conditions during which the wetland drains: (e.g., rainfall events) ____________________

____

____

D.5. GROUNDWATER SEEPAGE

Is there evidence of groundwater seepage at this watershed or beach area?

☐ yes ☐ no

If yes, please answer the following:

Proximity of groundwater seepage to recreational water: (approximate distance in feet) ____________________

Frequency of groundwater seepage: ____________________

Magnitude of groundwater seepage: (i.e., seepage volume) ____________________

Duration of groundwater seepage: ____________________

Are there any conditions under which groundwater seepage increases?: (e.g., rainfall events) ____________________

____

____

D.6. STREAMS OR NATURAL OUTFALLS

Are there streams or natural outfalls contributing to the fecal indicator concentrations at this watershed or beach area?

Streams: ☐ yes ☐ no Natural outfalls: ☐ yes ☐ no

Number of streams and natural outfalls: ____________________

Proximity of discharges to recreational water: (approximate distance in feet) ____________________

Frequency of discharges: ____________________

Magnitude of discharges: (i.e., stream or outfall volume) ____________________

Duration of discharges: ____________________

Are there any conditions under which stream or natural outfall discharge increases?: (e.g., rainfall events) ____________________

____

____

WORKSHEET E
AGRICULTURAL ANIMALS AND WILDLIFE

E.1. CAFOS/AFOS

Do the presence of animal feeding operations contribute to the fecal indicator concentrations at this watershed or beach area?

☐ yes ☐ no

If yes, please answer the following:

Type of impact: ☐ direct discharge ☐ indirect nonpoint source ☐ both direct and nonpoint sources

Are there any best management practices in place to control sources of runoff? ☐ yes ☐ no

Proximity of discharge to recreational water: (approximate distance in feet) ____________

Frequency of discharge: ____________

Does the frequency of discharge vary temporally? (e.g., monthly, by season) ____________

____

Magnitude of discharge: (e.g., overflow volume) ____________

Duration of discharge: ____________

Are there any conditions during which CAFO/AFO drainage increases? (e.g., rainfall events) ____________

____

What animals are present in the CAFO/AFO?

____

E.2. OTHER AGRICULTURAL RUNOFF

Are there other sources of agricultural runoff that contribute to the fecal indicator concentrations at this watershed or beach area?

☐ yes ☐ no

If yes, please answer the following:

Are there any best management practices in place to control sources of agricultural runoff? ☐ yes ☐ no

Proximity of runoff to recreational water: (approximate distance in feet) ____________

Frequency of runoff: ____________

Does the frequency of runoff vary temporally? (e.g., by season) ____________

____

Magnitude of runoff: (e.g., overflow volume) ____________

Duration of runoff: ____________

Are there any conditions during which other sources of agricultural runoff increase? (e.g., rainfall events) ____________

____

What animals contribute to the agricultural runoff?

____

E.3. PRESENCE OF ANIMALS AND WILDLIFE

Presence of Wildlife and Domestic Animals

Type	Degree of presence (low, mod, high)	Does the presence appear to correlate with bacteria results? (yes, no, don't know)	Describe further (include whether fecal droppings are seen and are a problem)
Geese			
Gulls			
Dogs			
Horses:			
Other (specify):			
Other (specify):			

E.4. DESCRIPTION OF OTHER FACILITIES

List facilities in the watershed or beach area that indicate presence homeless encampments or animals, such as dog parks.

Facility name/type	Location	Condition (good, fair, or poor)	Distance from beach (feet)	How might this facility contribute to water quality problems?

Comments/Observations:

DATA NEEDS JUSTIFICATION

SUMMARY OF POTENTIAL POLLUTANT SOURCES:

The information that is presented on the summary sheet provides an overview of the predominant fecal pollution at a particular site, that is, whether it is from human sources or not. This information will be useful in the short term for QMRA purposes, as it will determine the specific pathogens of interest. This information is also important for site-specific alternative criteria because it is used to determine which pathogens the site-specific QMRA will focus on. More specifically, the distance from and travel time to the recreational waterbody will be useful for determining the relative impact of a specific pollutant source on a specific site. Again, the sources of fecal contamination will determine the pathogens of interest, and that information will directly feed into the exposure assessment component of a QMRA.

SECTION 1: BASIC INFORMATION

It is assumed that the information in Sections 1A and 1B will be needed for record keeping purposes for any of the short- or longer-term potential uses of the survey. None of this information will be used for specific components of a QMRA analysis, but as indicated, would likely be needed to keep track of the areas to which the information collected later in the form refers.

Information related to sampling location: In the same way that the information in Section 1A is needed, it is also assumed that the information will be needed for record keeping purposes for any of the short- or longer-term potential uses of the survey. None of this information would be used for specific components of a QMRA analysis, but as indicated, would likely be needed to keep track of the areas to which the information collected later in the form refers.

WORKSHEET A

Information related to land use: These characteristics will be important for determining whether the predominant fecal pollution at a particular site is from human sources or not. If the land uses are residential, industrial, or commercial, it could be inferred that there is the potential for human contamination, whereas if the land use is all (or nearly all) agricultural, it may be that the primary source is nonhuman. This information would be useful in the short term for QMRA as it will determine the specific pathogens of interest. This information would also be useful in the longer term as part of the AWQC site classification (that is, which AWQC site type[s] is [are] most appropriate for a particular location—human impacted or impacted by other types of sources). Another potential long-term use is to develop site-specific criteria, in which case this information would be used to determine the pathogens on which the site-specific QMRA would be focused.

Information related to maps and photographs: These characteristics will be important for determining whether the predominant fecal pollution at a particular site is from human sources or not. If any of the anthropogenic-based map categories are present, it could lead to a presumption that there is the potential for human contamination. Similar to land use information, this information would be useful in QMRA for determining which pathogens would be of most interest (if human sources are not present, then viruses are likely less critical). The same type of logic would be used for longer-term purposes (site classification or development of site-specific criteria).

Information related to physical conditions: The information in this section is important for describing the beach area, but does not provide information directly related to QMRA. However, longshore or

nearshore currents could be considered as events that could potentially be managed if specific tides and/or currents correlated with increased exposures to indicators and pathogens. Again, this information would be useful in the exposure assessment component of QMRA.

WORKSHEET B

Information related to weather conditions: The information in this worksheet could be used in predictive models and/or by beach managers to identify conditions which are known to be of higher risk and to manage recreational areas (post, close, etc.), provided that this type of approach can be approved by the EPA. In these cases, the exposure assessment would result in increased levels of indicator (and presumably pathogen) exposures as compared to nominal conditions.

Information related to water quality sampling: These characteristics will be important for longer term uses, such as determining whether, or demonstrating that, site-specific criteria may be needed. For example, if there are no human sources, but there are bird sources, and the indicator levels are such that the new/revised AWQC appear to be not applicable for a specific site, these data would be needed to make that determination. Information related to unusual results would be used to demonstrate that specific hazardous events impact water quality.

Information related to advisories and/or closings The information in this section is important for describing the beach area, but do not provide information directly related to QMRA.

Information related to modeling: The information in this section is important for describing the beach area, but is not necessarily needed to provide information directly related to QMRA.

WORKSHEET C

Information collected on this worksheet will be used to identify human sources of fecal contamination that could impact a watershed or beach area. As indicated above, this information will be useful in the short term for QMRA as it will be used to determine the specific pathogens of interest and in the longer term as part of the AWQC site classification (that is, which AWQC site type[s] is [are] most appropriate for a particular location—human impacted or impacted by other types of sources). Another potential long-term use is to develop site-specific criteria, in which case this information would be used to determine the pathogens on which the site-specific QMRA would be focused. Specifically, these data would inform the exposure component of a QMRA, whether the purposes of that QMRA are short or long-term.

Information related to wastewater discharge: The information in this section is used to identify the potential water quality impacts of wastewater treatment facilities discharging to the waterbody of interest. In addition to being used to identify the presence of such a discharge, data are requested to characterize the proximity, discharge rate, travel time, and the frequency of any unintended discharge events. The type of treatment and disinfection employed are also important, as they will determine the relative level of indicators and pathogens that potentially could be present.

Information related to sewage overflows and CSOs: The information in this section is used to identify the potential water quality impacts of sewage overflows and CSOs. In addition to being used to locate the presence of such types of events, data are requested to characterize the proximity, discharge rate, travel time, and frequency of such events. The presence of these types of events would provide strong evidence of human-based fecal contamination.

Information related to unsewered areas and subsurface sewage disposal: This information will be important for determining whether the predominant fecal pollution at a particular site is from human sources or not. If unsewered areas or subsurface sewage disposal are close to a particular watershed, it may be difficult to consider the primary fecal pollution source as nonhuman.

Information related to marinas and sanitary facilities: These characteristics will be important for determining whether the predominant fecal pollution at a particular site is from human sources or not. If marinas or sanitary facilities are close to a particular beach, it may be difficult to consider the primary fecal pollution source as nonhuman.

Information related to bather load: These characteristics will be important for determining whether the predominant fecal pollution at a particular site is from human sources or not. This information could be used in QMRA to determine which pathogens are of highest concern in a particular waterbody. If human sources are considered important, the exposure assessment of QMRA will be used to account for human enteric viruses in addition to the pathogens that would be of interest from animal sources. Similarly, these data would be useful for longer term needs such as indicating the potential presence of human contamination in a waterbody (AWQC site classification). From a policy perspective, it is possible that QMRA could be used to identify a threshold bather density, such that any waterbody that exceeds some number (threshold) of recreators per volume of water would need to be classified as a human-impacted waterbody, even if all "upstream" sources are nonhuman.

WORKSHEET D

Information collected on this worksheet will be used to identify fecal contamination from stormwater, urban runoff, drains, streams, and wetlands that could impact a watershed or beach area. This information will be useful in the short term for QMRA as it will determine the specific pathogens of interest and in the longer term as part of the AWQC site classification (that is, which AWQC site type[s] is [are] most appropriate for a particular location—human impacted or impacted by other types of sources). Another potential long-term use is to develop site-specific criteria, in which case this information would be used to determine the pathogens on which the site-specific QMRA would be focused.

Information related to stormwater, urban runoff, and drains: The data collected in these sections will be used to document the impacts of stormwater, urban runoff, and drains on the waterbody of interest. Unlike data in the previous worksheet, these sources may be predominantly due to human or nonhuman sources. In each case, the proximity, frequency, magnitude, and duration will help to quantify the impacts of the sources. The question related to the known or suspected predominant source will be critical for use in QMRA related activities.

Information related to wetlands, groundwater seepage, and natural outfalls: The data collected in these sections will be used to document the impacts of wetlands, groundwater seepage, and natural outfalls on the waterbody of interest. Unlike data in the previous section, these sources may give a strong indication that contamination could be predominantly due to nonhuman sources. In each case, the proximity, frequency, magnitude, and duration will help to quantify the impacts of the sources. Like the previous section, the question related to the known or suspected predominant source will be critical for use in QMRA related activities.

WORKSHEET E

The information collected in this worksheet will be important for determining whether the predominant fecal pollution at a particular site is from animal sources and documenting that impact. If the animal sources are present and human sources are not, it could be inferred that the potential for human contamination is low. This information will be useful for the exposure assessment component of QMRA because the presence or absence of particular fecal sources will help to determine which pathogens will be of highest concern. This information will also be useful for longer-term uses (that is which AWQC site type[s] is [are] most appropriate for a particular location—human impacted or impacted by other types of sources). Another potential long-term use is to develop site-specific criteria, in which case this information would be used to determine the pathogens on which the site-specific QMRA would be focused.

<u>Information related to presence of CAFOs and AFOs and other agricultural runoff</u>: The information in these sections document the presence of agricultural animals in the watershed of interest. Data are also requested to document the proximity, frequency, magnitude, duration, and temporal nature of the runoff. Data indicating the predominant animals are also requested.

<u>Information related to presence of wildlife and domesticated animals</u>: Similar to the data from the previous sections in this worksheet, data requested in this section will be used to document the potential for animals and wildlife to impact the waterbody of interest. If data are available to link these sources with water quality impacts, those results should be highlighted and stressed. Due to limited health related data in the scientific literature on this topic, the extent to which these data can be useful for QMRA in a quantitative sense is not currently known. However, a lack of human-related inputs and documented impacts due to wildlife, would be valuable information for site characterization.

<u>Information related to other facilities</u>: The information in this section highlights potential nonhuman fecal contamination.

Appendix C: Example Sampling and Analysis Plan

Introduction

A sampling and analysis plan (SAP) describes the process for sampling and how the data will be analyzed, including quality assurance plans. A detailed SAP that is reviewed by others can help ensure that resources are allocated effectively. Data collection quality assurance procedures are also an important part of the SAP. The SAP also needs to include the indicator method that is associated with the water quality standard (WQS) being modified for site-specific alternative criteria. The SAP also needs to be representative of the meteorological and hydrological conditions in the watershed.

This appendix includes an example SAP for a water quality study that was conducted as part of a sanitary characterization. Initial information from the watershed suggested specific predominant sources of fecal contamination in the waterbody of concern. Furthermore, a sanitary survey was conducted to understand the potential sources of contamination in the waterbody and confirmed the initial information about the likely sources of contamination. Based on the results, water quality and fecal source studies were planned, developed, and carried out. The SAP describes the studies that were conducted to evaluate FIB and pathogenic microorganisms in the waterbody and the presumed source(s) of contamination.

The SAP in this appendix demonstrates that there is considerable flexibility in the experimental design for the water quality (fecal indicator, pathogen monitoring, source tracking) study included with the sanitary characterization step. In the SAP presented, the emphasis was on characterizing the presence and levels of human pathogens present at the beach site and in waters that were thought to have the potential to contribute fecal contamination to the beach site. This example SAP did include source tracking because the initial information suggested human contamination was likely. The example SAP provides an overview that can be taken into consideration during the iterative development of a SAP for water quality studies that are conducted as part of a sanitary characterization.

Boquerón SAP

Purpose of the Data Collection

The purpose of the EPA's Collection of Data for Calibrating the EPA's Quantitative Microbial Risk Assessment Methodology and Anchoring Results to an Epidemiology Study is to provide pathogen and indicator surveillance data in conjunction with an epidemiological study occurring during the summer of 2009 at Boquerón Beach, Puerto Rico. Potential effects on the recreational beach include a wastewater treatment plant (WWTP) outfall, two packaging plant outfalls, and other unknown fecal pollution sources.

Water samples from the beach and major fecal pollution source locations will be collected on Saturdays and Sundays for nine weeks beginning June 13, 2009. Water samples will be analyzed for a suite of pathogens and indicator organisms. The microbial water quality data will be used to anchor a quantitative microbial risk assessment (QMRA) model; therefore, concentrations of indicators and pathogens will be quantified when possible.

The information from this data collection effort will not be used for the development of a recreational water quality criteria (RWQC) value or for any other regulatory or rulemaking purposes. QMRA will be used to support the implementation of new criteria. Some of the methods used for pathogen analysis are research or experimental but have been determined to be the most viable in order to obtain the results.

Sampling Schedule and Locations

Water samples will be collected from each sampling site once on Saturday and once on Sunday, every weekend for nine consecutive weekends beginning June 13. The weekend of June 6 served as a training weekend for the field and laboratory staff, during which a dry run of an entire 2-day sampling event was conducted by all sampling personnel under the guidance of the Laboratory A Field Sampling Manager (for more detail on the training, see Section III).

A reconnaissance mission of the area was conducted during the week of May 25, 2009, to determine the greatest potential sources of pathogens to Boquerón Beach, after which exact sampling points were confirmed. Fecal contamination sources to be sampled include (1) the effluent of a publicly owned treatment work (POTW) (Prassa WWTP) before discharging to the outfall piping, (2) the effluent of a small package WWTP (Package Plant #2) before discharging to the outfall piping, and (3) the mouth of a lagoon into which multiple fecal contamination sources likely flow.

In addition, one combined sample will be collected from Boquerón Beach. This sample will be a combination of three sampling locations corresponding to the waist-depth sample points at the three transects being sampled in the EPA epidemiological study. Three field team members will each carry a carboy, wading into the water, following in the footsteps of field team members of the EPA's epidemiological study being conducted at the same time. Once the field team members have collected their samples, they will walk approximately 10 feet south in the water before walking straight back to the sand (out of the water). The EPA determined this was the least disruptive way to collect samples and minimize disruption to the sediment. In addition, collecting the samples immediately after the epidemiological study field team (approximately 8:00 a.m.) will provide the maximum time possible

between the morning and noon samples collected by the epidemiological study field team, minimizing disruption to their noon sample. Details about the sampling are included in Annex A.

Table C-1 includes descriptions of the sample locations for the study. Global positioning system (GPS) coordinates for each sampling site will be used during the first field event to verify that samples are collected from the correct locations. If, for some reason, the source sampling locations cannot be accessed during future sampling events (e.g., because of weather or a docked vessel), samples will be collected from a location as close as possible to the designated locations, and the alternate location will be noted on the Field Form.

Training Field Team Members

The weekend of June 6 served as a training weekend for the field and laboratory staff, during which a dry run of an entire 2-day sampling event was conducted by all sampling personnel under the guidance of the Laboratory A Field Sampling Manager. Both teams collected samples following the sampling protocol (provided in Annex A) on Saturday and Sunday at all four sampling sites. Samples were collected, placed on ice in coolers, and brought back to Laboratory B (grab samples) and Laboratory C (ultrafilters [UFs]). At Laboratory B, samplers were trained on the filtration method for the qPCR samples for *E. coli*, *Enterococcus*, and Bacteroidales (collected as a subsample from the grab sample bottles). For the filtration procedure, see Annex B. Samplers then prepared the UFs for further shipping (Section 5.E). At Laboratory C, samplers placed the UFs in the refrigerator until Monday, when they packaged the filters for shipping. Samplers also prepared and cleaned all the sampling equipment and supplies at Laboratory C.

For the rest of the sampling events, there will be two sampling teams, and each team will sample two sites per day for the duration of the project. One team will sample the Beach site and the POTW. The other team will sample the Lagoon site and the Package Plant. However, all sampling team members have been trained to sample each location and can switch teams if needed. Table C-2 provides the names and affiliations of the sampling team members. The names in bold are the team leaders. Additional members might be added in the future. If this occurs, they will be trained appropriately by one of the field team leaders.

Table C-1. Sampling locations.

Sample site number (from north to south)	Sample site name	Sample location description	Access	Comments
1	POTW – Prassa WWTP	Discharge point of the POTW	Drive to the POTW, access the site on foot	Samples will be collected at the discharge monitoring point, pumped directly from the plant's discharge chamber. The POTW effluent will first be pumped into large tanks (with new clean garbage bags as liners), sodium thiosulfate will be added to neutralize any chlorine present, and samples will be pumped from the tank through the filter apparatus. Grab samples will be collected as the tanks are filled, into sterile bottles containing sufficient sodium thiosulfate to neutralize the chlorine. Sample collection time will be following collection of the beach sample.
2	Boquerón Beach	The same three transects as used for the EPA epidemiological study (waist-depth point)	Drive to the beach, park in the public lot, access the beach to collect the sample on foot, wading from the beach	Samplers will arrive at the beach before 8:00 a.m. The sample from each transect will be collected immediately after the epidemiological study water samplers collect their waist-depth sample and exit the water at each transect. Three sampling team members will simultaneously wade into the water to the waist-deep sampling point of the transect. Each team member will submerge and fill a 20-liter (L) sterile carboy, aiming for a sampling depth of about 1 foot below the water surface, taking care to minimize disruption to the sediments. The samplers will then exit the transect by walking to their right (south) at least 10 feet (parallel to the beach) before turning back toward shore and walking out of the water. The carboys might be hauled away from the water with a small wagon. As the samples are collected in carboys from each transect, they will be transported out of the water and placed into a vehicle and hauled off the beach to a staging area for compositing and filtering. The staging area is along the road/parking lot near the mangroves between the beach site and the lagoon site. Discharge water from the filters will be discretely poured back onto the sand near the mangroves. No grab samples will be collected from the beach location.
3	Package Plant #2	Discharge point of the package plant	Drive to the package plant, access on foot	Samples will be collected at the discharge monitoring point, pumped directly from the plant's discharge chamber. The package plant effluent will first be pumped into large tanks (with new clean garbage bags as liners), sodium thiosulfate will be added to neutralize any chlorine present, and samples will be pumped from the tank through the filter apparatus. Grab samples will be collected as the tanks are filled, into sterile bottles containing sufficient sodium thiosulfate to neutralize the chlorine. The time of sample collection will be either before or after the lagoon sample, depending on the time of low tide.
4	Mangrove Lagoon	At the mouth of the lagoon	Boat (limited shore access because of mangroves)	At low tide, samples will be collected at the mouth of the lagoon, at the midpoint between the two channel markers. Samples will be pumped or grabbed (depending on the sample type) directly from the water and into the sample bottles and filter apparatus on the boat.

Table C-2. Field team members.

Name	Affiliation
Jane Doe	List affiliation
John Doe	List affiliation
Team member #1	List affiliation
Team member #2	List affiliation
Team member #3	List affiliation
Team member #4	List affiliation
Team member #5	List affiliation
Team member #6	List affiliation

Access/Health and Safety

Water samples from the WWTP will be collected from a trough at the plant. Field team members will wear appropriate clothing and protection devices if required by the plant (such as closed or steel-toed shoes and hard hats); however, plant operators have not yet requested this. Shore access to the lagoon sampling location is limited by the mangrove forest, and samples will be collected by boat. Boats will be operated by experienced drivers, and personal flotation devices will be available to all members of the sampling team.

Any field team member who wades out into the water to collect the beach sample will know how to swim and will not go out if conditions are dangerous (e.g., bad weather, rough water).

Sample Collection Procedures

A. Field Forms, Notebooks, and Photograph Log

The field teams will complete a Field Form at each sampling site during each sampling event (Annex C). Original copies of the forms will be saved and submitted to the EPA at the end of the project. Copies will be sent to the EPA periodically during the project when requested by the WAM. The forms will be completed in their entirety. If anything does not apply, “NA” will be noted on the form.

The samplers will use a field notebook to note any other observations made during a sampling event or any issues that occurred in addition to what was noted on the Field Forms.

Samplers will take at least one set of photographs at each sample point, to document the sample location and sample setup. If needed, samplers will take photographs of any unusual occurrences or issues that might affect sampling results. Samplers will keep a record of photographs in the field notebook, noting the time, date, location, and description of each image taken. Any photographs taken and copies of the photograph log will be submitted to the EPA after the last sampling event.

B. Sample Collection and Handling

1. Grab Samples

For those indicators with short hold times, grab samples will be collected in sterile 1-L Nalgene containers and split for multiple analyses at Laboratory B. The indicator analyses to be performed at Laboratory B include enterococci and *E. coli* by culture methods and filtering for future qPCR analyses (*Enterococcus* spp., *E. coli*, and Bacteroidales). Grab samples will be collected at the POTW, Package Plant, and Lagoon sites. Grab samples will not be collected at the Beach site.

For grab samples that will be processed in Laboratory B: 3 1-L bottles will be used for sample collection. Grab samples will be chilled in a cooler immediately after sampling and will be delivered to and processed at Laboratory B within a 6-hour hold time. Processing will be complete within an 8-hour hold time (i.e., petri dishes in incubators and membrane filters for qPCR in extraction tubes in a −20 degrees Celsius [°C] freezer). For each of the qPCR analyses (*Enterococcus* spp., *E. coli*, and Bacteroidales), the analysts will filter 100 mL through each of three 47-mm diameter with 0.4 µm pore size filters.

2. Ultrafilter Samples

The use of UFs (30,000 kilodaltons [kDa]) operated in the dead end mode was selected for use in this study because the goal of the study is to analyze pathogens and indicators from four sample sites of different matrices. This method has been used successfully by scientists from Laboratory A in previous studies where the focus is multiple pathogen concentration. This is a data collection effort and UF was determined to be the best way to concentrate samples for pathogen recovery and detection.

UF samples will be collected at all four sites (POTW, Package Plant, Lagoon, and Beach), with two filters used at each site for each set of samples. At each sample location, up to 200 L of water will be concentrated for pathogens and indicators using a dead-end ultrafiltration procedure similar to that developed by the Center for Disease Control and Prevention[32] and commonly used for outbreak investigations. Water samples will be concentrated on two Asahi REXEED-25S or 25SX dialyzers (also referred to as UFs) using two centrifugal pumps, pushing no more than 100 L water through each filter. For water samples that are collected at the Lagoon site by boat, the intake tubing will be tied to weighted line off the side of the boat and positioned about 1 foot below the water surface. Pumps will be run on 12 V batteries at the Beach and Lagoon sites and on electricity at the POTW and Package Plant sites, and there will be two dedicated pumps for each sampling location. These filters can be operated at 2 L–3 L/min, and at 2 L/min, the target volume can be sampled within 1 hour. However, filters can clog, and flow rates might diminish. Pumping will be terminated if the filters clog and the flow rate becomes less than 0.5 L/min or if significant backpressure builds up in the line. Only two filters will be used per sampling location for each sampling event, regardless of what volume of water is passed through the filter. The volumes filtered through each filter will be recorded by the sampling crew. Pumps will be disinfected after each use (chlorination followed by dechlorination).

After filtering, the UFs will be capped to seal the filters with the sample water inside the cartridge. The two UFs from one sampling site will be bagged together. The UFs will be placed in a cooler and then transferred to the Laboratory B refrigerator until they are shipped on ice Monday afternoon for priority

[32] Hill, V.R., A.L. Polaczyk, D. Hahn, J. Narayanan, T.L. Cromeans, J.M. Roberts, and J.E. Amburgey. 2005. Development of a rapid method for simultaneous recovery of diverse microbes in drinking water by ultrafiltration with sodium polyphosphate and surfactants. *Applied and Environmental Microbiology* 71:6878–6884.

overnight (Tuesday, no longer than 72 hours) delivery to Laboratory A. Filters must not freeze during shipment. Laboratory A will take the temperature of each filter immediately after receiving the shipment and will record this on the Chain of Custody Form (Annex D). Filters which have not maintained the proper temperature will not be analyzed and will be documented. The filters will be backflushed upon arrival at Laboratory A on Tuesday and subsamples of the UF concentrates will be shipped priority overnight on Tuesday (to arrive Wednesday morning) to Laboratory D for culturable virus analyses. Similarly, samples for norovirus analysis will be shipped to Laboratory E and frozen there and analyzed as a set when all samples have been received. Bacterial analysis at Laboratory A will begin Tuesday afternoon as soon as UF concentrates are ready.

Figure C-1 shows a flow chart that explains how the samples will arrive at each lab and what assays each lab will perform.

C. Sample Processing and Analysis

Water samples will be processed and analyzed for the list of pathogens and indicators included in Table C-3. Table C-3 also includes the laboratories that will be performing each analysis. Indicator methods will be the same as those being performed for the epidemiological study being conducted concurrently at Boquerón Beach and other EPA beach studies, where possible. Pathogen methods might not be standard methods—research methods or modified standard methods may be used to ensure the detection of pathogens and adapt to the sampling conditions.

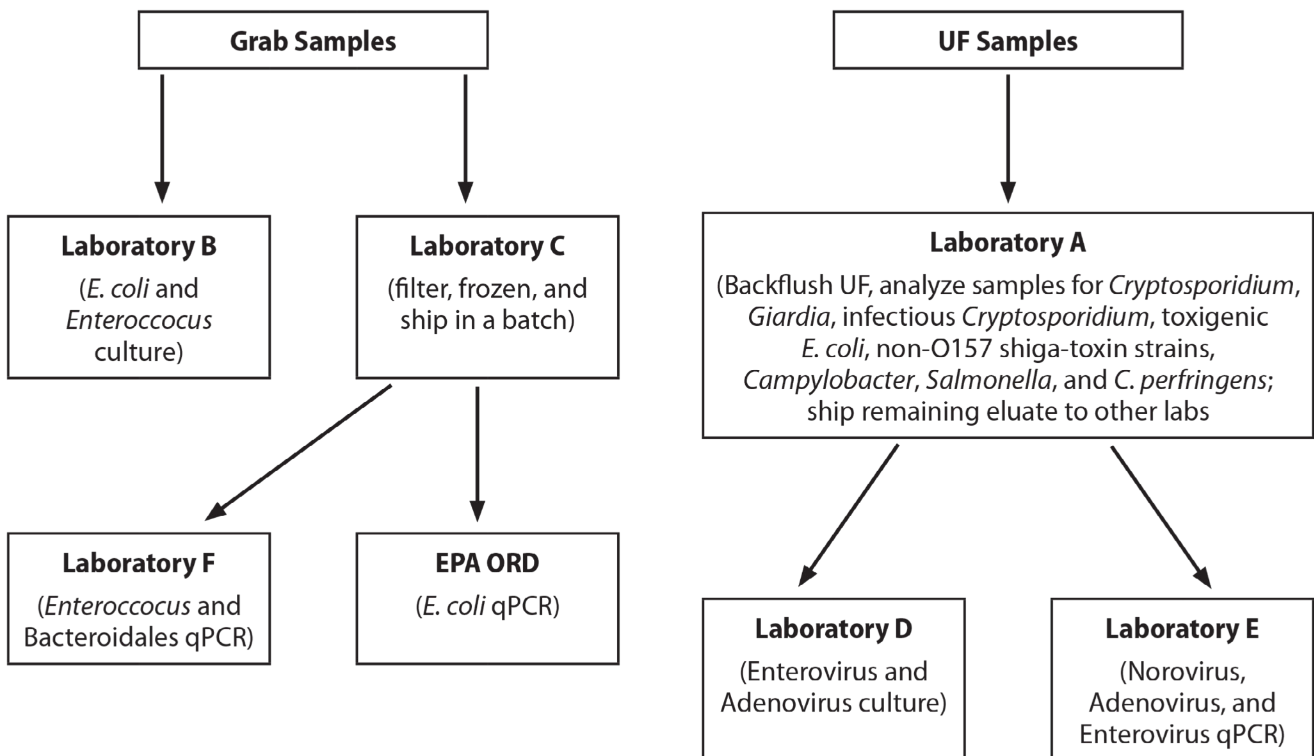

Figure C-1. Flow chart of laboratories.

Table C-3. Analytes and methods.

Microbe	Analysis method	Grab or UF concentrate or other	Equivalent original sample volume analyzed	Laboratory performing analysis
Pathogens				
Norovirus	qPCR	UF concentrate	Up to 20 L	Laboratory E
Adenovirus	Cell Culture Assay for Adenovirus with A549 Cell Line	UF concentrate	Up to 20 L	Laboratory D
Adenovirus	qPCR	Supernatant supplied from Laboratory D	To be determined	Laboratory E
Enterovirus	Enterovirus Culture Method Most-Probable Number Method	UF concentrate	Up to 20 L	Laboratory D
Enterovirus	CIPC-Qpcr	Supernatant supplied from Laboratory D	To be determined	Laboratory E
Cryptosporidium/ *Giardia*	EPA Method 1623	UF concentrate	Up to 10 L	Laboratory A
Infectious *Cryptosporidium*	Infectious *Cryptosporidium* Öocyst by cell culture IF antibody detection (Texas A&M Method)	UF concentrate	Up to 10 L	Laboratory A
Toxigenic *E. coli* O157:H7	Culture Method (IMS, CTSMAC, latex agglutination confirmation)	UF concentrate	1 L–10 L	Laboratory A
Toxigenic *E. coli* O157:H7	qPCR of UF Concentrate	UF concentrate	To be determined	Laboratory A
Non-O157 shiga-toxin strains	Culture Method (broth enrichment, plating on Rainbow agar and CTSMAC agar)	UF concentrate	1 L–10 L	Laboratory A
Non-O157 shiga-toxin strains	qPCR of UF Concentrate	UF concentrate	To be determined	Laboratory A
Campylobacter	Modification of ISO 17995 (2005), adapted to MPN test, use latex agglutination chips to confirm	UF concentrate	1 L–10 L	Laboratory A
Salmonella spp.	Modification of ISO 6340 (1995), adapted to MPN test, use enterotubes to confirm	UF concentrate	1 L–10 L	Laboratory A
Indicators				
Clostridium perfringens	MF on MCP agar (modified ICR method)	UF concentrate	Up to 1 L	Laboratory A
Enterococcus spp. and Bacteroidales	EPA's duplex TaqMan qPCR method for Enterococcus spp. and Bacteroides	Grabs filtered using 0.4 µm polycarbonate filters in Laboratory B	Up to 100 mL in duplicate	Laboratory F
Enterococci	EPA Method 1600	Grab	Up to 500 mL (in duplicate or triplicate)	Laboratory B
Male-specific F+ Coliphage	EPA Method 1602	UF concentrate	Up to 1 L	Laboratory A
E. coli	SM9221F (tube fermentation)	Grab	Up to 500 mL (in duplicate or triplicate)	Laboratory B
E. coli	EPA qPCR (23S)	Grabs filtered using 0.4 µm polycarbonate filters in Laboratory B	Up to 100 mL in duplicate	EPA ORD

1. *Grab Samples*

Three 1-L bottles of water will be collected as a grab sample at the Lagoon, POTW, and Package Plant sites. No grab samples are collected at the Beach site. These samples will be used for the following:

- Duplicate or triplicate 100 milliliter (mL) samples and dilutions will be analyzed locally at Laboratory B for *E. coli* using SM9221F and for *Enterococcus* spp. using EPA Method 1600 (membrane filtration and mEI agar).

- Triplicate 100 mL volumes will be filtered for *E. coli*, Bacteroidales and *Enterococcus* spp. qPCR following the EPA protocols. One filter will be used per analyte, following the protocol used in the EPA's epidemiological studies. Membrane filters will be frozen for the duration of the project. Soon after the last sampling event has occurred, the frozen filters will be shipped on dry ice and sent for deoxyribonucleic acid (DNA) extraction and qPCR at the appropriate subcontracted laboratory (Bacteroidales and *Enterococcus* spp. assays will be performed by Laboratory F,[33] and *E. coli* assays will be performed by the EPA ORD). The EPA standard operating procedure (SOP) for filtering water samples for qPCR and a quick reference guide (Annex B) has been provided to the Laboratory B staff for this project.

2. *UF Sample Processing and Analysis*

- UFs will be received at Laboratory A each Tuesday (shipped from Laboratory B each Monday). Upon receipt, the filters will be backflushed with 500 mL of backflush solution as described in Annex E. The resulting UF concentrates from the two filters collected at each site will be combined for a total volume of approximately 1 L per sample. The final concentrate volumes will be recorded on bench sheets. The concentrates will then be immediately split for the various analyses listed in Table C-3.
  - For some analyses, UF concentrates will require secondary concentration using centrifugation and analysis of the resuspended centrifugation pellet, or PEG precipitation or filtration through Centricon units. (This depends on the sample volume collected and if salt water or backflush solution needs to be rinsed away before analysis.)

- At Laboratory A, UF concentrate will be analyzed for *C. perfringens* using membrane filtration and mCP agar (hold times should have minimal effect on spores).

- At Laboratory A, a UF concentrate volume equivalent to 1 L–10 L original sample volume will be analyzed for each bacterial pathogen.
  - *Salmonella* spp. by enrichment broth culture (most probable number [MPN]) of UF concentrate (modification of ISO 6340 [1995]).
  - *Campylobacter* by enrichment broth culture (MPN) of UF concentrate (modification of ISO 17995 (2005).
  - *E. coli* O157 and toxigenic non-O157 *E. coli* by enrichment broth culture (MPN) of UF concentrate.

[33] Any filters which have not maintained the proper temperature will not be analyzed and will be documented.

- At Laboratory A, a UF concentrate volume equivalent to 1 L–10 L original sample volume will be analyzed for male-specific coliphage by modification EPA Method 1602 (Double Agar Layer).
- At Laboratory A, a UF concentrate volume equivalent to 20 L original sample volume will be centrifuged, and the resuspended centrifugation pellet will be split equally and analyzed for *Cryptosporidium* and *Giardia* by EPA Method 1623 (December 2005 version) and for infectious *Cryptosporidium*.
- A UF concentrate volume equivalent to up to 40 L original sample volume will be shipped priority overnight on Tuesday on ice to Laboratory D (to arrive Wednesday morning). Laboratory D will perform cell culture/MPN analyses for enteroviruses and adenoviruses. In addition, portions of the supernatant from these analyses will be sent to Laboratory E for PCR analyses. The shipment of the samples for Laboratory E will depend on the availability of their lab staff, but will likely be in a batch after the last sampling event.

Any remaining UF concentrate will be frozen and stored at Laboratory A, for potential repeat or alternate analyses during the course of study or for filtering for future qPCR analyses.

Recovery of Bacteria, Protozoa, and Viruses Using Dead-end Ultrafiltration

Dead-end Ultrafiltration was chosen for this project as the filters are easy to use, can accommodate large sample volumes (up to 100 L), allow capture of virus particles (molecular weight cutoff = 30,000 d), and are easily backflushed for recovery of target organisms. To assess recovery of target organisms of interest, the following study will be conducted at Laboratory A. A total of eight samples will be analyzed to develop performance data. The study design will be similar to that used in method performance assessments in Method 1622/23 (Annex F). The goal of this study is to assess the ability of the method to recover target organisms in reagent water and matrix samples.

Initial Precision and Recovery Experiments: Four filters will be spiked with 100 L reagent water seeded with *Salmonella* (~10^4/L) *Giardia* and *Cryptosporidium* (~10/L), and MS2[34] (~10^4/L). These organisms represent the target groups—bacteria, protozoan parasites, and viruses. The seed organisms will be spiked into 50 L of water and the samples filtered as described in the SAP for sample collection from a sanitized container. The filters will be spiked in the first half of sample collection, and followed by rinsing in the remaining 50 L reagent water. Samples will be backflushed and processed as described in the SAP for each method: *Giardia* and *Cryptosporidium* using Method 1623; MS2 using the double agar overlay; and *Salmonella* by two methods—the MPN as described in the SAP and by direct plating in XLD and SS agars. Samples for each target organism will be analyzed in replicate as total sample volume allows. The mean and standard deviation (SD) for each organism will be calculated.

Matrix Spike Samples: To examine the effect of the beach water matrix, Laboratory A will receive three 90-L samples of beach water from Boquerón Beach on the last two sampling rounds. The 90-L seawater samples will be seeded as described above with *Salmonella, Giardia, Cryptosporidium*, and MS2 and processed in the same manner. Recoveries and SDs will be calculated for the beach water matrix.

[34] MS2 is a virus that infects *E. coli*, a bacteriophage. It is a male-specific, also known as F+, bacteriophage. It is often used for laboratory seeding and recovery experiments and used as a positive control for F+ bacteriophage assays.

Method Blank: A method blank using 100 L reagent water will be sampled and processed as described to assess the presence/absence of any of the analytes in the sampling and analysis process.

D. Shipping

All samples shipped from Puerto Rico will be shipped with priority overnight delivery on the Monday after sample collection on a Saturday and Sunday, to arrive at the labs on Tuesday (no longer than 72 hours) morning. Samplers will compare the Chain-of-Custody form with the samples in the cooler to make sure all samples are present. Samplers will ensure compliance with U.S. Department of Transportation and the International Air Transport Association regulations regarding the transfer of hazardous substances and environmental samples. Samples being shipped from Laboratory B to Laboratory A will be shipped in standard coolers, double lined with trash bags. UFs will be placed in resealable bags inside the inner liner. Frozen ice packs will be placed in resealable bags between the inner and outer cooler liners, with sufficient ice packs used to keep samples properly chilled but not frozen. Both bags will be tied in a knot or with zip ties. A copy of the Chain of Custody form will be placed inside a resealable bag and taped to the inside of the cooler lid. Coolers will be taped shut and dropped off at the shipping center.

Samples that are shipped to Laboratory F and the EPA ORD (filters for qPCR analyses) will be shipped in coolers using dry ice. After filtering, each filter will be placed in an extraction tube, and all the tubes will be placed in a Styrofoam tube box in the freezer (−20 ^{o}C[35]) until ready for shipping (after the last sampling event in August). When ready to ship, the Styrofoam tube box will be placed inside a cooler. Up to (but not exceeding) 5 lbs of dry ice will be placed inside the cooler, surrounding the tube box. A Chain of Custody form will be placed inside a resealable bag inside the cooler. The cooler will be sealed and shipped with the proper labels on the outside of the box, indicating that the shipment contains dry ice.

Samplers will contact the labs that are receiving shipment to let them know what is being shipped and to give them a tracking number for each package.

Any field team member who wades out into the water to collect the beach sample will know how to swim and will not go out if conditions are dangerous (e.g., bad weather, rough water).

Annex A: Sampling Protocol Grab Sampling and Ultrafiltration

The sampling protocol listed below is to be used for collecting samples at Boquerón Beach, Puerto Rico, during the summer of 2009. There are four sample locations, and sample procedures vary slightly between each sample site. This protocol assumes that two sampling teams will each collect samples at two sites (four sites total) for two days in a row, every Saturday and Sunday for nine weeks. The teams will collect grab samples at each location except the beach and two UFs at each location. One team will collect samples at the Beach and the POTW sites, and the other team will collect samples at the Lagoon and the Package Plant sites. However, all team members will be trained to sample each location.

[35] The EPA qPCR SOP says filters can be stored at −20 °C or −70 °C. Laboratory B and Laboratory C can only store at −20 °C.

A.1 Equipment

Table C.A-1 is a list of recommended equipment that each sampling team should have with it for sampling in Boquerón. Before leaving for the sampling sites, the teams should review the list to make sure they have all items needed for sampling. This list might be adjusted slightly after the first sampling event occurs.

A.2. Sampling Setup

Packing of field supplies and equipment should be performed Friday and Saturday evenings using the equipment and supply list. As part of the sampling preparation, bleach and sodium thiosulfate solutions will need to be prepared for cleaning the equipment after use and for dechlorinating the water before sampling at the POTW and Package Plant.

Cleaning Equipment

Before sampling begins each weekend, each team needs to prepare a carboy of 1% bleach[36] (100 mL bleach + 9.9 L distilled water) (based on recommendations in the EPA's ICR Microbial Laboratory Manual (EPA 1996)[37] and a carboy of 0.05% sodium thiosulfate (0.5 g sodium thiosulfate in 10 L distilled water). The bleach may be used for up to a month as long as residuals remain high (chlorine level will be measured periodically using a chlorine test kit). These items will be used for cleaning the equipment after each sampling event (see the Post Sampling Procedures at the end of this appendix). The sodium thiosulfate carboy should be prepared fresh each weekend.

Dechlorination of water at the POTW and Package Pant

Eight small bottles of 38 g sodium thiosulfate should be prepared, mixed with a small amount of distilled water (just enough to dissolve) each week for dechlorination at the wastewater sites (POTW and Package Plant). Two bottles will be used at each of the two sites on each weekend day. This is likely to be more than what is needed to dechlorinate the wastewater. However, this ensures that enough sodium thiosulfate will be added in the event that chlorine levels are high (chlorine will be measured before and after adding the sodium thiosulfate).

A.3 Sampling Procedures

Sampling procedures vary slightly between each sample site. For the Lagoon site, samples will be collected directly from the lagoon water. For the beach site, carboy samples will be collected from three transects. These samples will be composited into two large sterile containers, where the water will be continually recirculated using sterile, dedicated bilge pumps. Sterile, dedicated centrifugal pumps will be used to pull water from this container and through the UFs. For the POTW and Package Plant sites, water will also be collected into large containers and continually recirculated using sterile, dedicated bilge pumps. This water will be dechlorinated using a sodium thiosulfate solution. After dechlorination, the sanitized, dedicated centrifugal pumps will be used to pull water from the container through the UFs. Grab samples will be collected during initial filling of the tank into sterile bottles containing sodium thiosulfate.

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[36] 10% bleach is not needed for this monitoring program

[37] U.S. EPA (U.S. Environmental Protection Agency). 1996. *ICR Microbial Laboratory Manual*. EPA/600/R-95/178. Government Printing Office, Washington, DC.

Table C.A-1. Equipment list for field teams.

Site 2 (Beach)	Quantity	?
Sampling		
20L Carboys	3	
Sample Tanks	2	
Sterile Tank Liners	2	
Bilge Pump	2	
12V Battery	1	
1-L Bottles	3	
Filtering		
5' Intake Tubing	2	
12V Pump	2	
12V Battery	2	
Filter Kit	1	
Filter	2	
5' Discharge Tubing	2	
Graduated Discharge Tanks	2	
Cleaning		
Bleach Carboy	1	
Na Thiosulfate Carboy	1	
Incidentals		
Tubing Cutters	1	
Labeling Tape	1 roll	
Duct Tape	1 roll	
Zip Ties	1 bag	
Sharpies/Pens	2	
Gloves	1 box	
Sanitizer	1	
Trash Bags	2	

Site 3 (Package Plant #2)	Quantity	?
Sampling		
High Flow Pump	1	
Extension Cord	1	
Sample Tanks	2	
Clean Tank Bags	2	
Bilge Pump	2	
Chlorine Kit	1	
38g Na Thiosulfate Bottle	2	
1-L Bottles	3	
Filtering		
5' Intake Tubing	2	
Electric Pump	2	
Filter Kit	1	
Filter	2	
5' Discharge Tubing	2	
Graduated Discharge Tanks	2	
Incidentals		
Tubing Cutters	1	
Labeling Tape	1 roll	
Duct Tape	1 roll	
Zip Ties	1 bag	
Sharpies/Pens	2	
Gloves	1 box	
Sanitizer	1	
Trash Bags	2	

Site 1 (PRASA POTW)	Quantity	?
Sampling		
High Flow Pump	1	
Extension Cord	1	
Sample Tanks	2	
Clean Tank Bags	2	
Bilge Pump	2	
Chlorine Kit	1	
38g Na Thiosulfate Bottle	2	
1-L Bottles	3	
Filtering		
5' Intake Tubing	2	
Electric Pump	2	
Filter Kit	1	
Filter	2	
5' Discharge Tubing	2	
Graduated Discharge Tanks	2	
High Flow Pump Fittings	2	
Incidentals		
Tubing Cutters	1	
Labeling Tape	1 roll	
Duct Tape	1 roll	
Zip Ties	1 bag	
Sharpies/Pens	2	
Gloves	1 box	
Sanitizer	1	
Trash Bags	2	
Teflon Tape	1 roll	

Site 4 (Lagoon)	Quantity	?
Sampling		
1-L Bottles	3	
No additional equipment needed. Lagoon water is pumped directly through the filter.		
Filtering		
Intake Tubing w/Dumb Bell	1	
12V Pump	2	
12V Battery	2	
Filter Kit	1	
Filter	2	
3' Discharge Tubing	2	
Graduated 5-gallon Buckets	4	
Incidentals		
Tubing Cutters	1	
Labeling Tape	1 roll	
Duct Tape	1 roll	
Zip Ties	1 bag	
Sharpies/Pens	2	
Gloves	1 box	
Sanitizer	1	
Trash Bags	2	

Sampling Procedure for the Lagoon Site

1. Arrive at the site by boat, using GPS coordinates if possible to make sure you are at the correct point. After first sampling, simply use channel markers to verify correct sampling location.

2. Fill out the Field Form.

3. Using label tape, label each filter with the Sample ID*. Leave space on the label for volume and times.

Sample ID:	__________
Filter Start Time:	__________
	__________
	__________

Example of a field label for filters

*The Sample ID incorporates the sample date and site; therefore, these are not listed separately on the sample labels.

4. Label the 1 L bottle for grab samples with Sample ID. Leave space on the label for times.

Sample ID:	__________
	__________

Example of a field label for grab samples

5. Pour the excess water out of the two UFs (through the end port and side port), if using a 25S (this is not necessary for the 25SX). Attach the molded DIN adapter and molded port cap as shown in Figure C.A-1.

6. Assemble two filtration systems as shown in Figure C.A-1, except do not connect the UF. Use new tubing from the box and cut to length with knife (discharge tubing may be reused).

7. Turn on the pumps and flush sample water through the sampling apparatus for 30 seconds and to prime the pumps.

8. Turn the valve to keep prime, turn off the pumps and reassemble the filtration system with the UFs as shown in Figure C.A-1. Remember to use hose clamps where directed on the diagram.

9. Turn on the pumps and begin pumping the sample water through the filtration system. Note the start filtration time on the Field Form.

10. During filtration, collect grab samples and place in cooler with ice. Record the sample collection time on the bottle label and the Field Form.

11. During filtration, direct discharge hoses to graduated 5-gallon buckets. Each time the bucket fills to the 10-L mark, switch out with a new bucket. Dump first bucket over the opposite side of the boat as the intake line. Keep track of how many 10-L volumes have been dumped. This procedure will determine the volume collected through the filters.
12. Stop filtration when one of the following has happened:
    a. 100 L has been filtered.
    b. The pressure increases and tubing begins to bulge.
    c. The flow rate has slowed to 0.5 L/min.
13. Record the end filtration time Field Form.
14. On the Field Form record the total volume filtered through each filter and write the volume on the filter label.
15. Replace the original caps on the UF side port and replace other end port with another molded cap.
16. Place both UFs in one 2-gallon resealable bag.
17. Place sample bottles and filters in a cooler with ice.
18. Return to the local lab for processing and shipping, or continue to the next sampling site.

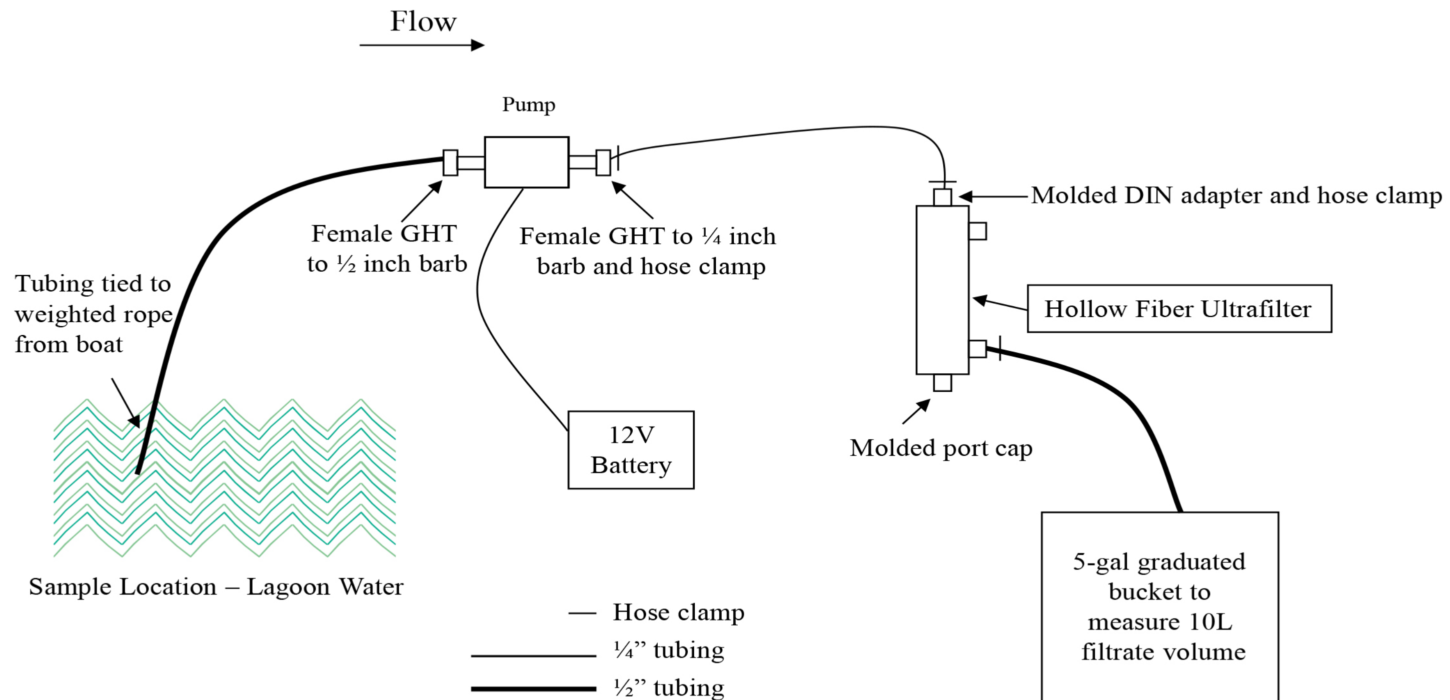


Figure C.A-1. Sampling setup at the lagoon site (there will be two identical setups, because two filters will be used).

Sampling Procedure for the POTW and Package Plant Sites

1. Arrive at the site and go to the effluent discharge sampling point.
2. Set up 30-gallon trashcans and insert clean garbage bag liners and sterile, dedicated bilge pumps.
3. Fill out the Field Form.
4. Test the effluent using the chlorine test kit. Note the chlorine level on Field Form.
5. Add the pre-measured sodium thiosulfate solution to the tanks.
6. Set up the pump according to Figure C.A-2.
7. Pump water into the tank until about 100-L level, making sure to continuously recirculate the water using the sterile, dedicated bilge pumps.
8. Collect grab samples as tanks fill and place in cooler on ice. Record the sample collection time on the bottle label and the Field Form.
9. Once the tank is full, turn off the pump.
10. Recheck the chlorine level and ensure it is zero.
11. Set up the sampling apparatus according to Figure C.A-2.
12. Using label tape, label each filter with the Sample ID. Leave space on the label for volume and times.
13. Label the 1-L bottles for grab samples with the Sample ID. Leave space on the label for times.
14. Pour the excess water out of two UFs (through the end port and side port), if using a 25S (this is not necessary for the 25SX). Attach the molded DIN adapter and molded port cap as shown in Figure C.A-2.
15. Assemble two filtration systems as shown in Figure C.A-2, except do not connect the UF.
16. Use new tubing from the box and cut to length with a knife (discharge tubing may be reused).
17. Turn on the pumps and flush sample water through the sampling apparatus for 30 seconds and to prime the pumps.
18. Turn the valves to hold the prime and turn off the pumps and reassemble the filtration system with the UFs as shown in Figure C.A-2. Remember to use hose clamps where directed on the diagram.
19. Begin pumping the sample water through the filtration system. Note the start filtration time on the Field Form.
20. Stop filtration when one of the following has happened:
    a. 100 liters have been filtered.

b. The pressure increases and tubing begins to bulge.

c. The flow rate has slowed to 0.5 L/min.

21. Record the end filtration time on the Field Form.

22. Record on the Field Form the total volume filtered through each filter and also write the volume on the filter label.

23. Replace the original caps on the UF side port and replace other end port with another molded cap.

24. Place both UFs in one 2-gallon resealable bag.

25. Place sample bottles and filters in a cooler with ice.

26. Dump or pump the discharge water back into the plant's discharge stream.

27. Return to the local lab for processing and shipping, or continue to the next sampling site.

Sampling Procedure for the Beach Site

1. Arrive at the beach at 7:30 a.m. to begin sampling at approximately 8:00 a.m. Drive your vehicle with sampling gear as close as possible to the transects, while taking care not to disturb the beach itself, lifeguards, or beachgoers.

2. In the back of the truck, prepare two 30-gallon tanks with sterile tank liner, sterile, dedicated bilge bumps, and clean intake tubing.

3. Begin filling out the Field Form.

4. Sampling begins immediately after the epidemiology study samplers finish sampling the first transect and exit the water to move down the beach.

5. All three sampling team members (each with one 20 L carboy) wade into the water at the first transect until approximately waist-deep (1 meter), lining up with the lifeguard tower right rail and palm tree.

6. Lower each carboy into the water approximately one foot below the water surface. When carboys are full, walk to the right (south) parallel to the shoreline approximately 10 feet and walk out of the water onto the beach. Using the wagon, transport the carboys to the truck. Pour 30 L into each of the two tanks.

7. Walk to the second transect and repeat Steps 4–6, piggy-backing the epidemiology study samplers as discretely as possible.

8. Walk to the third transect and repeat Steps 4–6, piggy-backing the epidemiology study samplers as discretely as possible.

9. Check the set-up of the two 30-gallon trash cans with sterile liners in the back of the vehicle. Pour approximately half of each carboy into the first lined trash can, and half into the second. Tie the top of the liners with zip ties. Load all sampling gear into the vehicle. Drive the vehicle off the beach, down the road, and pull off to the side near the nearby pathway to the mangrove lagoon for sample filtering.
10. Using label tape, label each filter with a Sample ID. Leave space on the label for volume and times.
11. Pour the excess water out of two UFs (through the end port and side port), if using a 25S (this is not necessary for the 25SX). Attach the molded DIN adapter and molded port cap.
12. Assemble two filtration systems as shown in Figure C.A-2, except without the UF. Use new tubing from the box and cut to length with a knife. Discharge tubing may be reused.
13. Turn on the pumps and flush sample water through the sampling apparatus for 30 seconds minute and to prime the pumps.
14. Turn the valve to *closed* to hold the prime and turn off the pumps. Reassemble the filtration system with the UFs as shown in Figure C.A-2. Remember to use hose clamps where directed on the diagram.
15. Begin pumping the sample water through the filtration system. Note the start filtration time on the Field Form.
16. Stop filtration when 90 L (the entire volume in the tank) has been filtered.
17. Record the end filtration time on the Field Form.
18. Record on the Field Form the total volume filtered through each filter and also write the volume on the filter label.
19. Replace the original caps on the UF side port and replace other end port with another molded cap.
20. Place both UFs in one 2-gallon resealable bag.
21. Place filters in a cooler with ice.
22. Dump the discharge water discretely onto the sand (not in the grassy area), which will flow through the mangroves and into the lagoon.
23. Return to local lab for processing and shipping, or continue to the next sampling site.

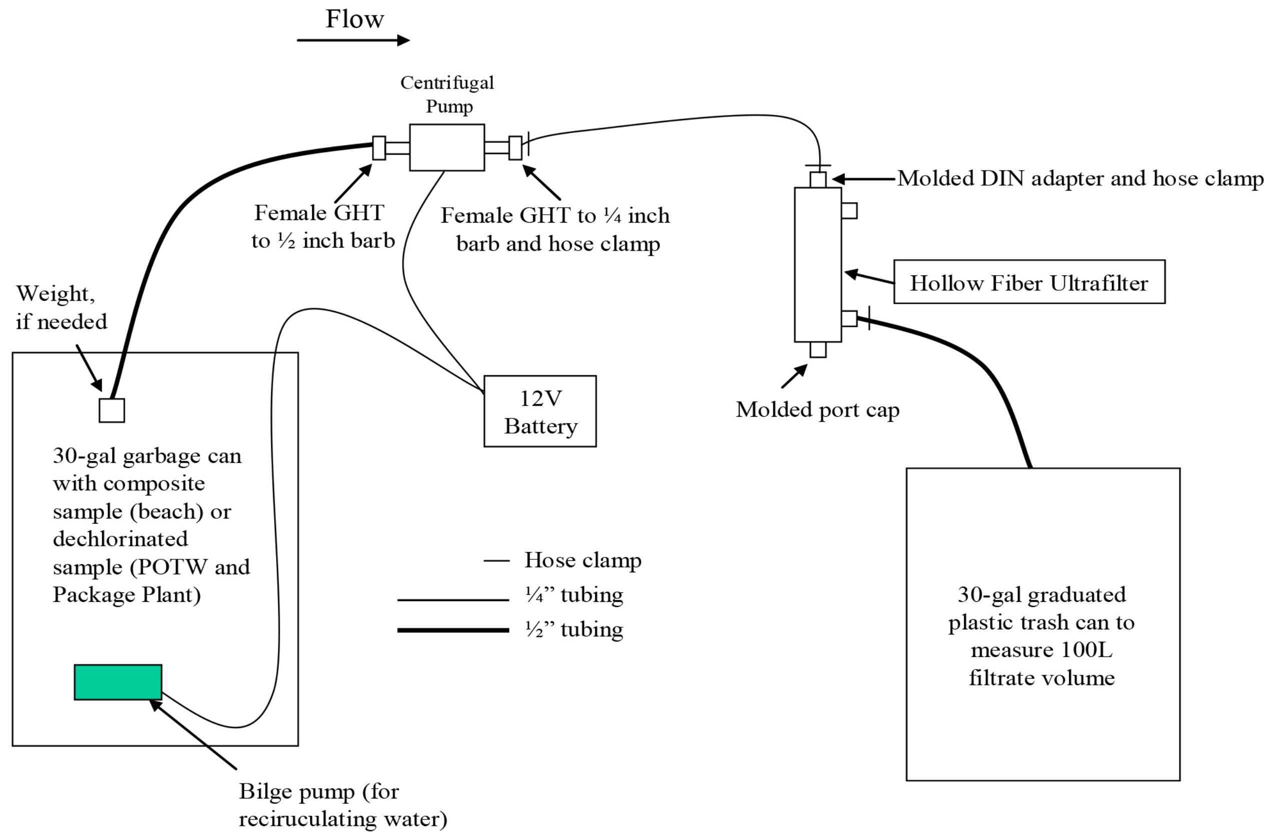


Figure C.A-2. Sampling setup at the beach, POTW, and package plant sites (there will be two identical setups, because two filters will be used).

Post-Sampling Procedures

This procedure applies to samples collected from all four sites.

1. Take **grab samples** to Laboratory B for processing each sampling day.

2. Transfer **UFs** from coolers to dedicated refrigerator at Laboratory C each sampling day.

3. At Laboratory C, hose down inside and outside of **trash cans** and set out to dry at the end of each sampling day.

4. At Laboratory C, clean up **chlorine kits** each sampling day.

5. **Dedicated Centrifugal Pumps**—pumps are dedicated for one field site and are to be cleaned in the field after each sampling.

    a. Keep the tubing from sampling attached to pump for this procedure.

    b. Place inlet and outlet tubing in the bleach water carboy; recirculate bleach solution for 5 minutes using the pump.

    c. Drain water from the pump and lines back into the carboy.

    d. Place inlet and outlet tubing in the sodium thiosulfate carboy; recirculate sodium thiosulfate solution for 5 minutes using the pump.

    e. Drain water from the pump and lines onto the ground.

    f. Discard tubing.

    g. Place the clean pumps in a new garbage bag.

    h. Tie up the bag and label with Site #, date and *Clean*.

6. **Carboys (there are three)**—for beach sampling; clean back at Laboratory B at end of each sampling day.

    a. Hose down outside of the carboys.

    b. Triple rinse inside with mild soap, rinse with tap water until all suds are gone.

    c. Pour 100 mL bleach into each carboy, fill with tap water to very top, let soak at least 30 minutes or overnight.

    d. Dump the bleach water down the drain, pouring water over carboy lids to disinfect those as well.

    e. Triple rinse with sodium thiosulfate solution from pump-cleaning carboys (250 mL of stock two percent sodium thiosulfate solution + 9,750 mL reverse osmosis [RO] water), rinse the carboy lids as well.

    f. Make sure bleach odor is completely gone from the carboys and lids.

g. Triple rinse each carboy with RO water.

h. Replace lids and set carboys aside for next beach sampling, label carboys as *Clean* and with the *date*.

7. **Dedicated Bilge Pumps**—pumps are dedicated for one field site and are to be cleaned back at Laboratory B at the end of each sampling day.

   a. Place pumps and most of wire in a 5-gallon bucket. Tape the end of the wire to the side of the bucket so that battery connections do not corrode.

   b. Pour in 1% bleach water to cover pumps and wires. Make sure water goes down inside pumps.

   c. Soak for five minutes in bleach water.

   d. Remove the pumps and shake out all excess bleach water.

   e. Transfer the pumps and wires similarly into another 5-gallon bucket of sodium thiosulfate solution from the pump-cleaning carboys (250 mL of stock 2% sodium thiosulfate solution + 9,750 mL RO water).

   f. Soak for five minutes in the sodium thiosulfate solution. Make sure the solution goes down inside pumps.

   g. Remove the pumps and shake out excess solution.

   h. Pour RO water over the pumps for final rinse.

   i. Place the clean pumps in resealable bags and label as *Clean* and with *Site #* and *Date*.

8. **Other important tasks**

   a. Sundays

      1. Check the charge on the batteries and begin recharging, if needed.

      2. Take inventory of supplies and e-mail Trisha Johnson ordering requests.

   b. Monday (or throughout week)

      1. Prepare eight sodium thiosulfate bottles for the next weekend sampling (38 g in enough RO water to dissolve, made in autoclaved plastic bottles).

   c. Friday

      1. Check chlorine level in the bleach carboy using the chlorine test kit.

      2. Prepare the fresh sodium thiosulfate carboy.

      3. Pack for weekend sampling.

Annex B: Quick Reference—Filtration of QPCR Samples at Laboratory B for Boquerón Study Summer 2009

Materials:

1. Dedicated workstation for water filtrations.
2. Water filtration apparatus, including disposable filter funnels and bases for 47-mm filter; vacuum manifold and trap with appropriate tubing connected to vacuum source (line, aspirator or electric pump).
3. Autoclaved extraction tubes with glass beads and tube rack.
4. Polycarbonate filters, 47-mm, 0.4-µm pore size.
5. PCR-grade water and autoclaved[38] squeeze bottles.
6. Pipettor and sterile disposable pipettes.
7. Two membrane filter forceps.
8. Ethanol, 95%.
9. Flame source.
10. Permanent marking pen for labeling tubes.
11. Bench sheets/lab notebook and black pen.
12. Styrofoam tube box for storage of extraction tubes in a freezer.
13. Freezer at ≤ −20 °C.[39]

Procedures:

1. Remove the funnel, place a polycarbonate filter on the filter base and replace the funnel.
2. Shake the water sample bottle vigorously to suspend the bacteria uniformly. For lagoon samples, filter 100 mL. For wastewater samples, filter at least 50 mL and up to 100 mL if possible. Filter each sample in **triplicate** (3 filters).
3. Thoroughly rinse the sides of the funnel twice with PCR-grade water using an autoclaved squeeze bottle.
4. Turn off the vacuum and remove the funnel from the filter base.
5. Using sterile forceps, fold the filter on the filter base into a cylinder with the sample side facing inward and insert the filter into an extraction tube with glass beads. (Note: Handle the filter

[38] Standard times for autoclaving will be used, as written in EPA's methods.
[39] The EPA qPCR SOP says filters can be stored at −20 °C or −70 °C. Laboratory B and Laboratory C can only store at −20 °C.

with the forceps at the edges; do not touch the portion of the filter exposed to the water sample. Try not to allow the filter to contact the outside edges of the extraction tube.)

6. Cap the extraction tube tightly and label it.
7. Replace used filter funnels with new sterile funnels. Repeat Steps 1–6 until all water samples have been processed.
8. For filter blanks, filter 50 mL of PCR-grade water using one new sterile filter funnel per filter blank.
9. Samples must be filtered within six hours and placed in the −20 °C freezer within eight hours of sample collection time.
10. Perform **one** filter blank for every **six** sample filters processed.

Label each tube with the Sample ID (e.g., 7JUN09-1A), and label field blanks as date-FB (e.g., 7JUN09-FB); if you do more than one filter blank in a day, label as date-FB1, -FB2, etc.

Annex C: Field Form

Field Form for EPA QMRA Study—Boquerón Summer 2009

Date: ________________ Members of Field Crew: ____

Weather Conditions: ____

Sampling Information:

Asahi Kasei Rexeed Dialyzer Type (circle one)	Sampling Site[1]	Filter Field ID[2]	Start Filtration Time	End Filtration Time	Volume Filtered (L)	Total Chlorine Residual *before* Dechlorinaton (mg/L)	Total Chlorine Residual *after* Dechlorination (mg/L)[3]	Grab Sample Collection Time	Carboy Sample Collection Time at each Beach Transect (T3, T2, T1)
25S or 25SX									
25S or 25SX									
Comments/Observations:									
25S or 25SX									
25S or 25SX									
Comments/Observations:									

[1]Sites: **1** (Prassa WWTP) **2** (Boquerón Beach) **3** (Package Plant #2) **4** (Lagoon)
[2]For Filter ID, use Date (e.g. 06JUN09), Hyphen (-), Site # (1-4), and letter "A" for Saturday event or letter "B" for Sunday event.
[3]Each tank of 100 L wastewater effluent is dechlorinated with 38 g sodium thiosulfate.

Annex D: Sample Chain of Custody Form

Clancy Environmental Consultants, Inc.		CHAIN OF CUSTODY RECORD
PROJECT:	EPA QMRA Study--Boqueron Summer 2009	Destination
Facility (shipped from):	CECIA-IAUPR	CEC
Courier:		20 Mapleville Depot
Airbill #		St. Albans, VT 05478

Sample Collection Point ID	Sample ID (Filter Field ID)	Sample Date	Sample Collection Start Time	Sample Collection End Time	Volume Filtered (L)	Sample Type	Temperature (°C) of Filter upon Receipt in Lab (for CEC use)	LAB ID (for CEC use)	Remarks
1									
1									
2									
2									
3									
3									
4									
4									
1									
1									
2									
2									
3									
3									
4									
4									

Dispatched by: *(Signature)*	Date	Time	Received by: *(Signature)*	Date	Time

Annex E: Instructions for Preparing the Backflush Solution

The solution will be used for backflushing UFs collected for the Puerto Rico Project. Sixteen 500-mL bottles of solution need to be prepared every Monday following the steps below. The backflush solution consists of 0.5% Tween 80, 0.01% sodium polyphosphate, and 0.001% antifoam Y-30 emulsion, filter sterilized.

1. Prepare a Stock Solution weekly by adding the following ingredients to 440 mL of distilled water:

   a. 0.8 g sodium polyphosphate

   b. 0.08 mL (80 µL) antifoam Y-30 emulsion

   c. 40 mL Tween 80

   Place the stock solution on a stir plate with a stir bar. The solution will take approximately 25 minutes to completely dissolve.

2. Add 30 mL stock solution to 470 mL distilled water in the funnel of a 0.2 µm disposable sterilizing filter funnel. Use the pipette to thoroughly mix.

3. Attach the funnel to vacuum and filter sterilize.

4. Repeat Steps 1–3 another 15 times for a total of sixteen 500 mL bottles of Backflush Solution, using a fresh sterilizing filter funnel each time.

5. Label each bottle with *Backflush Solution*, *Date*, and the preparer’s initials.

6. Refrigerate the bottles.

Additional Prep

(Items needed by Tuesday morning of each week)

1. Wash and autoclave eight squat-form 500 mL plastic graduated cylinders.

2. Wash and autoclave eight 1 L glass or plastic bottles.

Instructions for Ultrafilter Backflush

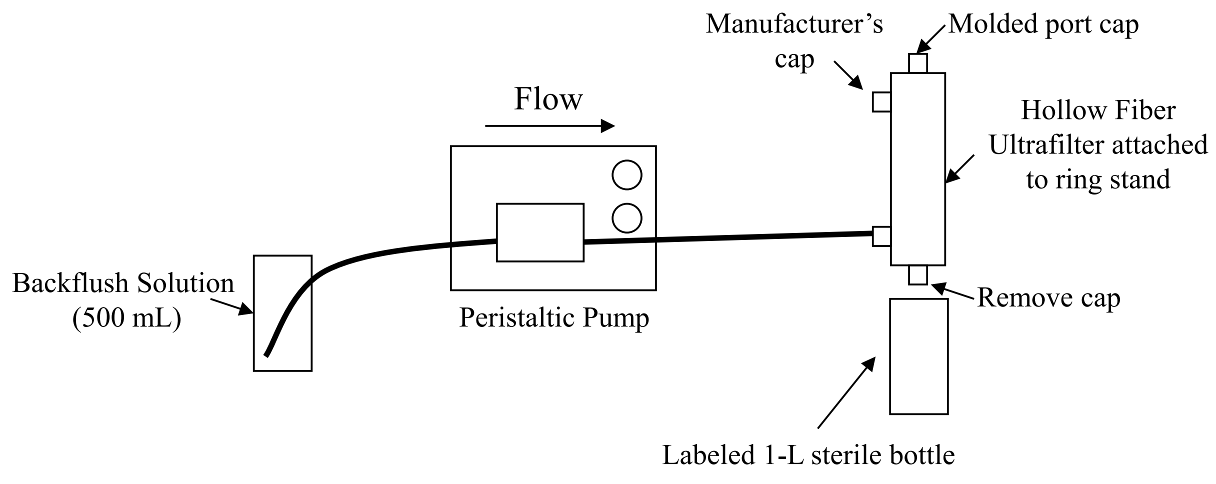
**Sixteen UFs collected for the Tetra Tech Puerto Rico Project will arrive at Laboratory A every Tuesday morning. Sixteen 500 mL bottles of backflush solution will be prepared before the arrival of the filters. UFs will be backflushed according to the procedure described below.*

Materials:

- Underpads
- Gloves
- Ringstand and clamp
- Peristaltic pump
- L/S 36 tubing
- 16 bottles sterile Backflush Solution
- 8 sterile 500 mL grad cylinders
- 8 sterile 1 L glass or plastic bottles
- Benchsheet
- Black marker and black pen
- Scalpel or tubing cutter
- Labeling tape
- Trash can
- Large biohazard bag

1. UFs from the Beach and Lagoon sites will be backflushed at the bench; UFs from the POTW and Package Plant sites will be backflushed in the Biological Safety Cabinet (BSC).
2. Wear gloves.
3. Waste generated from the Beach/Lagoon UF processing can go in the regular trash. Waste generated from the POTW/Package Plant UF processing must go in a biohazard bag to be autoclaved and then discarded.
4. Lay down an underpad and set up a backflush station with a peristaltic pump and a ringstand as shown in Figure C.E-1.
5. Label a sterile 1 L glass or plastic bottle with the Lab ID, Date, and Field ID. Leave a space to record volume.
6. Remove two bottles of backflush solution from fridge and take to bench/BSC.
7. Remove a bag of two UFs (having the same Field ID and Lab ID) from the cooler/fridge and take to the bench/BSC.
8. Record the Field ID and Lab ID on the benchsheet.
9. Remove one filter from the bag and open both *side* ports to dump excess water trapped in the filter housing into a waste beaker. Replace the side port caps.
10. Place the UF in the ringstand, remove the bottom port cap (blue end of UF), and position the UF over the 1 L sterile glass bottle as shown in Figure C.E-1.
11. Remove the bottom side port cap from the UF.
12. Cut fresh L/S 36 tubing to the needed length (using a scalpel or tubing cutter) and attach to side port of UF as shown in Figure C.E-1.
13. Be careful to keep the end of the tubing that will go into backflush solution clean; try not to lay it down on the work surface.

14. Load the tubing into peristaltic pump head.
15. Place the other end of tubing into a fresh bottle of backflush solution.
16. Check the direction of the pump flow and turn the pump on at a rate of 650 mL/min to push the backflush solution into the UF. Water should begin to drain from the UF into the glass bottle.
17. On the benchsheet, note the time backflushing begins.
18. Tilt the bottle of the backflush solution as it empties to drain as much solution from the bottle as possible.
19. When the flow from the UF slows from a steady stream to a drip, immediately turn off the pump.
20. Reverse the pump flow direction and turn on the pump for 10 seconds. This will release the pressure that has built up in the UF.
21. Remove the UF from the setup, cap all ports, and discard the UF.
22. Using the same 1 L glass bottle and the same tubing, repeat Steps 9 through 21 for the other UF in the bag (which has the same Sample ID).
23. Using the sterile graduated cylinder, measure the total volume of UF concentrate combined from the two filters. (The easiest way to do this is to measure 500 mL of the UF concentrate, pour it into one of the *empty* backflush solution bottles, measure another 500 mL and pour it into the other *empty* backflush solution bottle, and measure the last small volume in the grad cylinder. Then pour the entire amount back into the 1 L bottle).
24. Record the total volume on the 1-L bottle label and on the benchsheet.
25. Place the UF concentrate in the refrigerator and continue with backflushing the remaining UFs.
26. Each pair of UFs will require a new grad cylinder, 1-L glass bottle, two bottles of backflush solution, and new tubing.
27. Wipe down the work area with bleach water and dispose of the underpad. Turn on the ultraviolet (UV) light in the BSC for 30 minutes.



L/S 36 tubing

Figure C.E-1. UF backflush setup.

Annex F: Laboratory Standard Operating Procedures and Protocols

Laboratory procedures specific to this study: microbiological measurement protocols and procedures not included herein (reference methods published under copyright) will be maintained by the respective laboratories and made available for review on request.

- Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples

Pathogens:

- Norovirus qPCR
  - Dr. Schwab's Norovirus PCR Procedure and Bench Sheet
- Adenovirus Culture Method
  - Laboratory D's Virus Cell Culture Manual
  - Laboratory D's Cell Culture and Assay for Adenovirus with A549 Cell Line
  - Laboratory D's Quantitation of Total Culturable Virus Data Sheets
- Adenovirus qPCR Method
  - Dr. Schwab's Adenovirus PCR Procedure and Bench Sheet
- Enterovirus Culture Method Most-Probable Number Method (Supernatant Used for Enterovirus PCR Method
  - USEPA Manual of Methods for Virology. Chapter 14: Concentration and Processing of Waterborne Viruses by Positive Charge 1MDS Cartridge Filters and Organic Flocculation
  - USEPA Manual of Methods for Virology. Chapter 15: Total Culturable Virus Quantal Assay
  - Laboratory D's Quantitation of Total Culturable Virus Data Sheets
- Enterovirus Using RT-PCR Method for Enterovirus PCR
  - Dr. Schwab's Enterovirus PCR Procedure and Bench Sheet
- *Cryptosporidium* and *Giardia* using EPA Method 1623 for *Cryptosporidium* and *Giardia* in Water by Filtration/IMS/FA
  - EPA Method 1623: *Cryptosporidium* and *Giardia* in Water by Filtration/IMS/FA
  - EPA Method 1622/23 Benchsheet and Text
  - EPA Method 1623 Hemacytometer Data Sheet for *Cryptosporidium* or *Giardia* and Text
  - EPA Method 1622/1623 Spiking Suspension Enumeration Form: *Cryptosporidium* or *Giardia* and Text

    - EPA's Method 1622/1623 *Cryptosporidium* Report Form and Text
    - EPA's Method 1622/1623 *Giardia* Report Form and Text
- Infectious *Cryptosporidium* Culture/Research Method
    - EPA's ICR Microbial Laboratory Manual, Section VII
    - AwwaRF 3021 Detection of Infectious *Cryptosporidium* in Filtered Drinking Water SOP (Giovanni, 2006)
    - EPA's ICR Microbial Laboratory Manual, Appendix VII-4, *Cryptosporidium* Report Form
- Toxigenic *E. coli* O157:H7 and Non-O157 Shiga-Toxin Strains Culture/Research Method
    - Laboratory A's Toxigenic *E. coli* O157:H7 and Non-O157 Shiga-Toxin Protocol and Benchsheet
- *Campylobacter* Culture/Research Method
    - Laboratory A's *Campylobacter* Protocol and Benchsheet
- *Salmonella* spp. Culture/Research Method and qPCR Research Method
    - Laboratory A's *Salmonella* Culture Protocol and Benchsheet
- Toxigenic *E. coli* O157:H7 and Non-O157 Shiga-Toxin Strains and *Salmonella* spp. qPCR Research Methods
    - *E. coli* O157:H7 and Non-O157 Shiga-Toxin Strains and *Salmonella* spp. qPCR Protocols and Benchsheets (including sample processing protocol, DNA extraction, and PCR run protocols
        - Laboratory A's Preliminary Information on Quantification of Bacterial Pathogens Using qPCR
        - Laboratory A's qPCR Centrifugation Log
        - Laboratory A's qPCR Filtering Log
        - EZ1® DNA Investigator Handbook (QIAGEN, 2009)
        - Rotor-Gene® Q—Pure Detection (QIAGEN, 2009)

Indicators:

- *Clostridium perfringens*
    - EPA's ICR Microbial Laboratory Manual, Section XI
    - Laboratory A's SOP for Detecting *Clostridium perfringens* in Water and Wastewater Using the Membrane Filter Method
    - Laboratory A's *Clostridium* Laboratory Benchsheet

- Male-specific (F+) Coliphage
    - EPA Method 1602: Male-specific (F+) and Somatic Coliphage in Water by Single Agar Layer Procedure
    - Laboratory A’s Standard Operating Procedure for Production and Enumeration of Male Specific Coliphages
    - Laboratory A’s Standard Operating Procedure for Male Specific (F+) Coliphages in Water—Single Agar Layer
    - Laboratory A’s Initial Precision and Recovery Trials for Male-Specific Coliphage Using U.S. EPA Method 1602
    - Laboratory A’s Method 1602—Male Specific Coliphage in Water
- *E. coli* SM9211F
    - Laboratory B’s E. coli SM9211F Protocol
- Enterococcus EPA Method 1600 (Membrane-Enterococcus Indoxyl-β-D-Glucoside Agar Membrane Filter Method for Detection of Enterococci)
    - Method 1600: Enterococci in Water by Membrane Filtration Using Membrane-Enterococcus Indoxyl-β-D-Glucoside Agar
    - Laboratory B’s Method 1600 Protocol
- Enterococcus & Bacteroides EPA Duplex qPCR Research Method
    - EPA’s *Enterococcus* spp. and Bacteroidales Method described in Laboratory E’s Rapid, PCR Based Method for Measuring Total *Enterococcus* spp. and Bacteriodales in Water Samples
    - Laboratory F’s Example Setup Page
    - Laboratory F’s Example Run Report

Appendix D: Water Quality Standards Submission Checklist

This appendix provides a checklist of additional information that is useful to include in the WQS submission that includes alternative WQC. Following this list will help the EPA determine if the WQS submission is based on sound science and protective of the designated use, as required in 40 CFR § 131.6 and 40 CFR § 131.11. Users provide substantiation for the identified decision points at each step. This list complements the information in the EPA's Water Quality Standards Handbook chapter on "Procedures for Review and Revision of Water Quality Standards," which describes the process for submitting and the EPA review of WQS.[40] A reevaluation of the sanitary survey once every three years (e.g., at the time of a state or Tribal triennial review) should be sufficient to confirm that the sources do not substantially differ from those characterized in Steps 1 and 2.

Information Provided by the TSM User

Step 1: Understanding Sources Impacting the Watershed

Decision Point: Is there clear evidence that nonhuman fecal sources impact and possibly dominate the waterbody?

_______QMRA sanitary survey results[41]

_______Existing and historical management practices

_______Past monitoring data

_______Existing information on fecal source identification and tracking

_______Survey of wildlife, agricultural, and domestic animals

_______Future planning and zoning information (if available)

_______Maps

_______Hydrological data

_______Meteorological data

Step 2: Conduct Water Quality Study

Decision Point: Are nonhuman fecal contamination sources confirmed to be the predominant fecal source affecting the waterbody?

_______Sampling and analysis plan for conducting the water quality study

_______Select reference pathogens based on source

_______FIB densities (geometric mean [GM] of enterococci in marine water and enterococci or *E. coli* in freshwater) in the waterbody and presumed fecal source

_______Reference pathogen densities in the waterbody and presumed fecal source

_______Fecal source identification

_______Other studies that provide information on sources

Step 3: Human Health Risk Evaluation

Decision Points:

Identify water quality value and associated target illness rate to adjust for nonhuman fecal sources.

[40] https://www.epa.gov/wqs-tech/water-quality-standards-handbook

[41] A Sanitary Survey form developed for recreational water locations for which QMRAs will be conducted can be found in TSM Appendix B. The EPA has also provided a mobile application for conducting beach sanitary surveys: https://www.epa.gov/beach-tech/sanitary-surveys-recreational-waters#epa

How do the water quality and illness estimates compare to the water quality and illness targets of the applicable water quality standard?

Does this information support deriving alternative water quality criteria for the waterbody?

_______FIB and reference pathogen data collected at the site and in sources that impact the site (from Step 2)

_______Limits of quantification for the pathogen methods

_______Sources of variability and uncertainty

_______Additional site-specific assumptions

_______Site-specific factors and data not included and the justification for not including those factors (e.g., temporal shifts in loading)

Step 4: Calculate alternative WQC GM that corresponds with the selected benchmark illness level

Decision Points:

Are the decisions and risk assessment assumptions transparently documented?

Determine the magnitude, duration, and frequency elements to be included in the criteria.

Are the proposed criteria scientifically defensible and protective of the designated use?

_______Transparent calculation of the GM that corresponds with the target illness rate.

_______Transparent documentation and justification of any parameters used in the reverse QMRA by either pointing to the TSM or, if using values not discussed in the TSM, pointing to information supporting those parameters.

_______Calculate the statistical threshold value (STV) and beach action value (BAV) corresponding to the GM and include the duration component of the alternative criteria and transparent documentation of calculations.

Appendix E: Example Code for Step 3: Approach 1

This appendix provides an annotated code example for Step 3, Approach 1 forward QMRA. The code estimates the mean and median illness levels for ambient recreational waters for which measured pathogen densities are available. The code may be adapted for other pathogens by defining and appropriately including parameters. It is recommended that this code be utilized by experienced programmers after a careful reading of its syntax and structure with reference to the appropriate equations described in the TSM. This is an example and should not be considered as a tool. A key for the annotated code is presented in Table E-1 and the definitions and sources for variables for the Step 3, Approach 1 forward QMRA is presented in Table E-2.

Table E-1. Key for annotated code.

KEY
BOLD—Actual R code (R available at http://www.r-project.org/)
PLAIN—Comments for guidance

Table E-2. Step 3, Approach 1 forward QMRA: definitions and sources for variables in equations.

In-text variable	Code Variable X represents the pathogen of interest	Definition
C_{rp}^{M}	testdata$_X Note: _X refers to different pathogen names	Measured density of reference pathogen in waterbody (pathogens/L).
V	rand_vol	Volume of water ingested (L)
μ_{rp}		Estimated dose of reference pathogen ingested, computed directly as product of C_{rp}^{M} and V (number of pathogens)
P_{ill}^{rp}	pill_x	Probability of illness from a specific reference pathogen (unitless)
P_{ill}	Tpill	Estimated probability of illness (unitless)
f_{d-r}^{rp}	pill_bpa, pill_exp, pill_hyp, pill_hyp_cj	Dose-response functions
$p_{ill\|inf}^{rp}$	drp_X$c_pill Note: _X refers to different pathogen names	Probability of illness following infection (unitless)

```
#!/usr/bin/Rscript
##########################################################################################
### Example code for QMRA Approach 1 based on measured pathogen densities
### Example relies on an R dataframe named "testdata" which contains data on simultaneously ###### measured pathogen densities in separate columns in units of pathogens/L
### The "testdata" dataset is not provided. The dataset name and column names will differ in the user's dataset.
##########################################################################################
rm(list=ls()); # Removes all previous objects from the R environment
set.seed(1); ## Ensures numerical replicability of simulation
```

```
###############################################################################
### The code below checks whether or not required libraries "hypergeo" have been installed previously.
### If not, the code downloads and installs the required packages first.
###############################################################################
list.of.packages <- c("hypergeo","MASS")
new.packages <- list.of.packages[!(list.of.packages %in% installed.packages()[,"Package"])]
if(length(new.packages)) install.packages(new.packages)
require("hypergeo");
require("MASS");
########################################
### Dose Response Functions
########################################
####################################################################
### The following lines define generic dose-response functions
### which may be applied to one or more pathogens when supplied
### with the appropriate parameters. These functions implement
### the formulas provided in Table 2-5.
####################################################################
############################################
### Beta Poisson Dose Response Function
############################################
pill_bpa <- function(a, b, c_pill, d){
	return((1-(1+d/b)^-a)*c_pill)
};
###########################################
### Exponential Dose Response Function
###########################################
pill_exp <- function(r, c_pill, d){
	return((1-exp(-r*d))*c_pill)
};
###############################################
### Confluent Hypergeometric Expression
### of Beta Poisson Dose Response Function
###############################################
pill_hyp <- function(alpha, beta, c_pill, d){
	return((1-genhypergeo(alpha, alpha+beta, -d))*c_pill)
};
#############################################################
### Confluent Hypergeometric Expression
### of Beta Poisson Dose Response Function
### Implements Teunis 2018 / 2020 Bayesian parameter method
#############################################################
### Function sample w_z jointly samples w and z (transformed parameters) from posterior distribution expressed as ###
bivariate normal
sample_w_z<-function(means, vcov,n){
  return(mvrnorm(n, mu = means, Sigma = vcov)) ### inputs are vector of means and the variance covariance matrix
}
## Function hyp1F_t20 computes probability of infection based on w, z parameter distribution using the confluent
## hypergeometric function
hyp1F1_t20<-function(inf_wz_dist,d){
  w<-inf_wz_dist[,1]; z<-inf_wz_dist[,2] ### Inputs are joint w,z distribution and dose
  u<-exp(w)/(1+exp(w)); v<-exp(z)
  alpha<-v*u; beta<-v*(1-u)
  pill<-c()
  for (i in 1:n_samp){
```

```
  p<-1-genhypergeo(alpha[i],alpha[i]+beta[i],-d)
  p<-pmin(1,p)
  p<-pmax(0,p)
  pill<-c(pill,p)
 } # end of for
 return(pill)}

### Function cpill_t20 computes conditional probability of illness based on w, z parameter distribution
cpill_t20<-function(ill_wz_dist,d){
 w<-ill_wz_dist[,1]; z<-ill_wz_dist[,2] ### Inputs are joint w,z distribution and dose
 u<-exp(w)/(1+exp(w)); v<-exp(z)
 r<-v*u; eta<-v*(1-u)
 cpill<-c()
 for (i in 1:n_samp){
  p<-1-(1+d/eta[i])**-r[i]
  p<-pmin(1,p)
  p<-pmax(0,p)
  cpill<-c(cpill,p)
 }# end of for
 return(cpill)}

### Function pill_hyp_t20 computes median probability of illness combining probability of infection and condititonal
#### probability of illness given infection
pill_hyp_t20 <- function(inf_wz_dist,ill_wz_dist, dose){
 p_inf<-hyp1F1_t20(inf_wz_dist,dose) ### Inputs are joint w,z distribution for both infection and illness, and dose
 c_pill<-cpill_t20(ill_wz_dist,dose)
 p_ill<-c_pill*p_inf
 return(median(p_ill))
};
##### Dose Response Parameters for Pathogens of Interest
##############################################################
########################################################################
### The following lines define and save the dose response parameters
### for each of the pathogens occurring at the site in "data frames".
### These values are drawn from Table 2-5.
########################################################################
###################################################################
### Salmonella Dose Response Parameters (for Beta Poisson DRF)
### c_pill is actually a uniformly distributed range of values
### (0.17 to 0.4). The mean value has been used.
###################################################################
drp_sal <- data.frame(a=0.3126, b=2884, c_pill=0.285);
##############################################################
### Adenovirus Dose Response Parameters (for Confluent Hypergeometric DRF
### with dose dependent conditional probability of illness)
##############################################################
drp_ade <- data.frame(alpha=5.11, beta=2.8, r=0.41, eta=6.53);
#####################################################################
### Cryptosporidium Dose Response Parameters (for Exponential DRF)
### c_pill in the line below is actually a uniformly distributed
### range of values (.3 to .7). The mean value has been used.
#####################################################################
drp_cryp <- data.frame(r=0.09, c_pill=0.5);
####################################################################
### Giardia Dose Response Parameters (for Exponential DRF)
### c_pill in the line below is actually a uniformly distributed
```

```
### range of values (.2 to .7). The mean value has been used.
##################################################################
drp_gia <- data.frame(r=0.0199, c_pill=0.45);
##################################################################
### Norovirus Dose Response Parameters
### (Confluent Hypergeometric Expression of Beta Poisson DRF)
### using the Teunis dose dependent dose response function
### Both sets of parameters are specified as distributions per Teunis 2020)
##################################################################
drp_nor <- list(inf_wz_means=c(-0.608, 0.194),inf_wz_vcov=matrix(c(1.79, -1.03, -1.03, 2.54),2),ill_wz_means=c(1.74,
1.82),ill_wz_vcov=matrix(c(5.55, -0.708, -0.708, 4.64),2));
##################################################################
### Campylobacter jejuni Dose Response Parameters
### (Confluent Hypergeometric Expression of Beta Poisson DRF
### using the Teunis dose dependent dose response function
### Both sets of parameters are specified as distributions per Teunis 2018)
##################################################################
drp_camp <- list(inf_wz_means=c(-0.177,0.054),inf_wz_vcov=matrix(c(1.303,-0.041,-0.041,1.070),2),ill_wz_means=c(-
2.744,-4.89E-3),ill_wz_vcov=matrix(c(1.337, 0.010, 0.010, 0.993),2));
########################################################################################
### The following line specifies the number of simulations that are to be performed
########################################################################################
numsim <- 1000; ### number of simulated swim events
n_samp<-1000; ### number of samples from Bayesian posterior parameter distribution for Teunis 2018 and 2020 DRFs
##################################################################
###################################
### Defining matrix dimensions
###################################
tpill <- matrix(0, numsim); # tpill is the matrix that will contain multiple estimates of the estimated total probability of
illness from a single swim event
############################################################
### Sample from normalized parameter posterior distributions
### for DRFs based on Teunis 2018 and 2020
###(Generate w,z distributions for infection and illness)
############################################################
nor_inf_wz_dist<-sample_w_z(drp_nor$inf_wz_means, drp_nor$inf_wz_vcov,n_samp)
nor_ill_wz_dist<-sample_w_z(drp_nor$ill_wz_means, drp_nor$ill_wz_vcov,n_samp)
camp_inf_wz_dist<-sample_w_z(drp_camp$inf_wz_means, drp_camp$inf_wz_vcov,n_samp)
camp_ill_wz_dist<-sample_w_z(drp_camp$ill_wz_means, drp_camp$ill_wz_vcov,n_samp)
################################################
### Beginning the swim event simulation loop
################################################
for (i in 1:numsim){
		#### Randomly select pathogen densities and volume ingested for each swim event####
		rand_row <- floor(runif(1, 1, nrow(testdata)+1)); # Randomly picks a row of data from the data file

# (this is known as bootstrap sampling with replacement)
		rand_vol <- rlnorm(1,  -3.98, 1.43);  # Randomly picks an ingested volume from a lognormal distribution
							# with characteristics of ln mean=-3.98 and ln sd=1.43.  (Dufour, 2017)
		# Units adjusted to output volume in L. These parameter values are from Table 2-8.
	##########################################################################
	### The following lines compute the individual probability of illness
	### from each pathogen in the data during the simulated single swim event
	##########################################################################
	### Estimate Salmonella Risk (Equation 1B and 1C)
		pill_sal <- pill_bpa(drp_sal$a, drp_sal$b, drp_sal$c_pill, testdata$sal_c[rand_row]*rand_vol);
```

```
### Estimate Adenovirus Risk (Equation 1B)
        pill_ade <- pill_hyp(drp_ade$alpha, drp_ade$beta,drp_ade$r, drp_ade$eta,
testdata$aden_c[rand_row]*rand_vol);
    ### Estimate Cryptosporidium Risk (Equation 1B and 1C)
        pill_cryp <- pill_exp(drp_cryp$r, drp_cryp$c_pill, testdata$cryp1623[rand_row]*rand_vol);
    ### Estimate Giardia Risk (Equation 1B)
        pill_gia <- pill_exp(drp_gia$r, drp_gia$c_pill, testdata$giar1623[rand_row]*rand_vol);
    ### Estimate Norovirus Risk (Equation 1B)
        pill_nor <- pill_hyp_t20(nor_inf_wz_dist,nor_ill_wz_dist, testdata$tcv[rand_row]*rand_vol);
    ### Estimate Campylobacter Risk (Equation 1B)
        pill_camp <- pill_hyp_t20(camp_inf_wz_dist,camp_ill_wz_dist, testdata$camp_c[rand_row]*rand_vol);
    ### The following line computes illnesses per 1000 events from all pathogens in the data during the simulated
single swim event (Equation 1D)
        tpill[i] <- 1000*(1-(1-pill_sal)*(1-pill_ade)*(1-pill_cryp)*(1-pill_gia)*(1-pill_nor)*(1-pill_camp)); #
Estimate and Save Total Risk from all pathogens

} # End the simulation loop
### Output summary statistics relating to the total probability of illness from swimming ###
print(paste("The estimated mean illnesses per 1000 events using Approach 1 is", round(mean(tpill, na.rm=T), digits=2)));
### Output summary statistics relating to the total probability of illness from swimming ###
print(paste("The estimated median illnesses per 1000 events using Approach 1 is", round(median(tpill, na.rm=T),
digits=2)));
### Output summary statistics relating to the total probability of illness from swimming ###
print("Summary statistics of the estimated illnesses per 1000 events using Approach 1 are:")
summary(tpill, na.rm=T)

df<-data.frame(unclass(summary(tpill, na.rm=T)), check.names = FALSE, stringsAsFactors = FALSE)

write.csv(df,"Yourpath/YourFilename.csv", row.names = FALSE)
```

Appendix F: Example Code for Step 3: Approach 2

This appendix provides an annotated code example for Step 3, Approach 2 forward QMRA. The code estimates the mean and median illness levels for ambient recreational waters that are contaminated by seagull sources. The code may be adapted for other animal sources by defining and appropriately including parameters for those sources. It is recommended that this code by utilized by experienced programmers after a careful reading of its syntax and structure with reference to the appropriate equations described in the TSM. This is an example and should not be considered a tool. In this example code all the references to FIB refer to enterococci, however, it can be adapted to other FIB, such as *E. coli*. A key for the annotated code is presented in Table F-1 and the definitions and sources for variables for the Step 3, Approach 2 forward QMRA is presented in Table F-2.

Table F-1. Key for annotated code.

KEY
BOLD—Actual R code (R available at http://www.r-project.org/)
PLAIN—Comments for guidance

Table F-2. Step 3, Approach 2 forward QMRA: definitions and sources for variables in equations.

In-text variable	Code variable X represents the pathogen of interest	Definition
C_{rp}^{S}	Crp_X Note: _X refers to different pathogen names	Estimated density of reference pathogen in the waterbody derived from the Source S (pathogens/L)
C_{FIB}	testdata$ent	Measured waterbody density of enterococci using a culture method (CFU/L)
R_{FIB}^{S}	Rfib	Density of enterococci (CFU/L or CFU/g) in the source S
R_{rp}^{S}	Rrp_X Note: _X refers to different pathogen names	Density of reference pathogens (number of pathogens or genomes/L or number of pathogens or genomes/g) in the Source S
μ_{rp}^{S}	dose_x Note: _X refers to different pathogen names	Estimated dose ingested of reference pathogen derived from the Source S (number of pathogens)
p_{rp}^{S}	Inf_x$prp Note: _X refers to different pathogen names	Fraction of human infectious pathogenic strains in the Source S (unitless)
I_{rp}^{S}	Inf_x$irp Note: _X refers to different pathogen names	Prevalence of infection in the nonhuman Source S (unitless)
V	rand_vol	Volume of water ingested (L)
P_{ill}^{S}	tpill	Estimated probability of illness from a specific source of contamination (unitless)
P_{ill}^{RP}	drp_X$c_pill Note: _X refers to different pathogen names	Probability of illness from a specific reference pathogen (unitless)

```
#!/usr/bin/Rscript
#####################################################################################################
### Example code for QMRA approach based on measured FIB density assuming seagulls as
### the source of fecal contamination. Example relies on an R dataframe named "testdata" which
### contains data on measured FIB density in units of CFU/L.  The "testdata" dataset is not provided. The dataset name and
column names will differ in the user's dataset.

#####################################################################################################

rm(list=ls()); # Removes all previous objects from the R environment
set.seed(1); ## Ensures numerical replicability of simulation

####################################################################################
### The code below checks whether or not required libraries have been installed previously.
### If not, the code downloads and installs the required packages first.
####################################################################################
list.of.packages <- c("hypergeo","MASS")
new.packages <- list.of.packages[!(list.of.packages %in% installed.packages()[,"Package"])]
if(length(new.packages)) install.packages(new.packages)
require("hypergeo");
require("MASS");

##########################################
### Dose Response Functions
##########################################

########################################################################
### The following lines define generic dose-response functions
### which may be applied to one or more pathogens when supplied
### with the appropriate parameters. These functions implement
### the formulas provided in Table 2-5.
########################################################################

##############################################
### Beta Poisson Dose Response Function
##############################################
pill_bpa <- function(a, b, c_pill, d){
        return((1-(1+d/b)^-a)*c_pill)
};

################################################################
### Confluent Hypergeometric Expression
### of Beta Poisson Dose Response Function
### Implements Teunis 2018 / 2020 Bayesian parameter method
################################################################
### Function sample w_z jointly samples w and z (transformed parameters) from posterior distribution expressed as ###
bivariate normal
sample_w_z<-function(means, vcov,n){
 return(mvrnorm(n, mu = means, Sigma = vcov)) ### inputs are vector of means and the variance covariance matrix

## Function hyp1F_t20 computes probability of infection based on w, z parameter distribution using the confluent
## hypergeometric function
hyp1F1_t20<-function(inf_wz_dist,d){
 w<-inf_wz_dist[,1]; z<-inf_wz_dist[,2] ### Inputs are joint w,z distribution and dose
 u<-exp(w)/(1+exp(w)); v<-exp(z)
 alpha<-v*u; beta<-v*(1-u)
```

```
 pill<-c()
 for (i in 1:n_samp){
  p<-1-genhypergeo(alpha[i],alpha[i]+beta[i],-d)
  p<-pmin(1,p)
  p<-pmax(0,p)
  pill<-c(pill,p)
 } # end of for
 return(pill)}

### Function cpill_t20 computes conditional probability of illness based on w, z parameter distribution
cpill_t20<-function(ill_wz_dist,d){
 w<-ill_wz_dist[,1]; z<-ill_wz_dist[,2] ### Inputs are joint w,z distribution and dose
 u<-exp(w)/(1+exp(w)); v<-exp(z)
 r<-v*u; eta<-v*(1-u)
 cpill<-c()
 for (i in 1:n_samp){
  p<-1-(1+d/eta[i])**-r[i]
  p<-pmin(1,p)
  p<-pmax(0,p)
  cpill<-c(cpill,p)
 }# end of for
 return(cpill)}

### Function pill_hyp_t20 computes median probability of illness combining probability of infection and conditional
probability of illness given infection
pill_hyp_t20 <- function(inf_wz_dist,ill_wz_dist, dose){
 p_inf<-hyp1F1_t20(inf_wz_dist,dose) ### Inputs are joint w,z distribution for both infection and illness, and dose
 c_pill<-cpill_t20(ill_wz_dist,dose)
 p_ill<-c_pill*p_inf
 return(median(p_ill))
};

##############################################################
##### Dose Response Parameters for Pathogens of Interest
##############################################################

##############################################################################
### The following lines define and save the dose response parameters
### for each of the pathogens occurring in the source (seagulls)
### in "data frames". These values are drawn from Table 2-5.
##############################################################################


###################################################################
### Salmonella Dose Response Parameters (for Beta Poisson DRF)
### c_pill is actually a uniformly distributed range of values
### (0.17 to 0.4). The mean value has been used.
###################################################################
drp_sal <- data.frame(a=0.3126, b=2884, c_pill=.285);



################################################################
### Campylobacter jejuni Dose Response Parameters
### (Confluent Hypergeometric Expression of Beta Poisson DRF
### using the Teunis dose dependent dose response function)
```

```
#################################################################

drp_camp <- list(inf_wz_means=c(-0.177,0.054),inf_wz_vcov=matrix(c(1.303,-0.041,-0.041,1.070),2),ill_wz_means=c(-
2.744,-4.89E-3),ill_wz_vcov=matrix(c(1.337, 0.010, 0.010, 0.993),2));

#################################################################################
### The following lines define and save the infectivity parameters for
### each of the pathogens occurring in the source (seagulls) in "data frames"
### The parameters irp and prp are from Table 2-4 and Table 2-3 respectively.
#################################################################################
inf_sal <- data.frame(prp=(75+100)/2/100, irp=0.165); # Defining prp (fraction of human infectious pathogenic strains from
###source) and irp (prevalence of infection in source) for Salmonella
inf_cam <- data.frame(prp=(54+100)/2/100, irp=0.165); # Defining prp and irp for Campylobacter


#######################################################################################
### The following line specifies the number of simulations that are to be performed
#######################################################################################
numsim <- 1000 # Number of simulations
n_samp<-1000; ### number of samples from Bayesian posterior parameter distribution for Teunis 2018 and 2018 DRFs

### Defining matrix dimensions ###
tpill <- matrix(0, numsim) # tpill is the matrix that will contain multiple estimates of the estimated total probability of
### illness from a single swim event


############################################################
### Sample from normalized parameter posterior distributions
### for DRFs based on Teunis 2018 and 2020
###(Generate w,z distributions for infection and illness)
############################################################

camp_inf_wz_dist<-sample_w_z(drp_camp$inf_wz_means, drp_camp$inf_wz_vcov,n_samp)
camp_ill_wz_dist<-sample_w_z(drp_camp$ill_wz_means, drp_camp$ill_wz_vcov,n_samp)

##################################################
#### Beginning the swim event simulation loop
##################################################
for (i in 1:numsim){

        ### The following lines randomize (for each swim event) the FIB density, Volume ingested, FIB density in source,
# and Pathogen density in source ###
                rand_row <- floor(runif(1, 1, nrow(testdata)+1)); # Randomly selects a row of data from the data file (this
#is known as bootstrap sampling with replacement)

                rand_vol <- rlnorm(1,  -3.98, 1.43);  # Randomly picks an ingested volume from a lognormal distribution
                                                      # with characteristics of ln mean=-3.98 and ln sd=1.43.  (Dufour,
        #2017)
                # Units adjusted to output volume in L. These parameter values are from Table 2-8.
        ### The pathogen and FIB density parameters used in this block are from Table 2-2 and 2-7 ###
                Rfib <- 10^runif(1, 4.114, 9.447); # Randomly simulates the density of fecal indicator enterococci in source
# in CFU/g. If you are using E. coli, change the values of this parameter.
                Rrp_sal <- 10^runif(1, 2.3, 9.0);          # Randomly simulates the density of Salmonella in source
#organisms/g
                Rrp_cam <- 10^runif(1, 3.3, 6.0);          # Randomly simulates the density of Campylobacter in source
#organisms/g
```

```
	### The following lines estimate the dose ingested during the swim event ###
		Crp_sal <- (testdata$ent[rand_row]/Rfib)*Rrp_sal;		# EQUATION 2A: Calculates the density of
### Salmonella in water in pathogens/L.
		dose_sal <- Crp_sal*inf_sal$prp*inf_sal$irp*rand_vol;  # EQUATION 2B: Dose calculation for Salmonella.
		Crp_cam <- (testdata$ent[rand_row]/Rfib)*Rrp_cam;		# EQUATION 2A: Calculates the
### density of Campylobacteria in water in pathogens/L.
		dose_cam <- Crp_cam*inf_cam$prp*inf_cam$irp*rand_vol;  # EQUATION 2B: Dose calculation for
### Campylobacteria

	### The following lines compute the individual probability of illness from each pathogen in the source during the
 ### simulated single swim event
		pill_sal <- pill_bpa(drp_sal$a, drp_sal$b, drp_sal$c_pill, dose_sal);
	# EQUATION 2C: Estimate Salmonella Risk
		pill_cam <- pill_hyp_t20(camp_inf_wz_dist,camp_ill_wz_dist, dose_cam);
	# EQUATION 2C: Estimates Campylobacter Risk

	### The following line computes illnesses per 1000 events from all pathogens found in the source during the
###  simulated single swim event

		tpill[i] <- 1000*(1-(1-pill_sal)*(1-pill_cam)) # EQUATION 2D: Estimate and Save Total Risk from all
###  pathogens
} # End the simulation loop

print(paste("The estimated mean illnesses per 1000 events using Approach 2 is", round(mean(tpill, na.rm=T),
digits=2)));# Output summary statistics relating to the total probability of illness from swimming

print(paste("The estimated median illnesses per 1000 events using Approach 2 is", round(median(tpill, na.rm=T),
digits=2)));# Output summary statistics relating to the total probability of illness from swimming

### Output summary statistics relating to the total probability of illness from swimming ###
print("Summary statistics of the estimated illnesses per 1000 events using Approach 1 are:")
summary(tpill, na.rm=T)

## Output summary stats in a file

df<-data.frame(unclass(summary(tpill, na.rm=T)), check.names = FALSE, stringsAsFactors = FALSE)

write.csv(df,"YourFilePath/YourFileName.csv", row.names = FALSE)

#######################################################################################################
############################################################################
### ADDITIONAL NOTE: If the summary outputs an error message, it is most likely because the simulated dose has
exceeded the range of R's confluent hypergeometric function. ###
### Rerunning the code usually corrects this problem. The reason this occurs is attributable to the high-end values for
pathogen densities in the source.
### The greater the numbers of simulations that are run, the more likely the extreme case will be simulated
############################################################################################
```

Appendix G: Reverse QMRA Gull Case Study

Introduction

This appendix describes a reverse QMRA case study that implements the reverse QMRA approach discussed in Section 2.2.2.4.5 of this TSM and corresponds to Step 4 in the process to estimate FIB levels for waterbodies predominated by nonhuman fecal sources (Section 3.4). The EPA constructed this illustrative example to assist users with application of this TSM. The scenarios characterized in this case study are hypothetical waterbodies predominantly affected by sea gull feces or by a nonfecal source of indicator (i.e., no enteric pathogen contribution). Soller et al. (2010b) presented a QMRA-based analysis characterizing the influence of multiple sources of enterococci (human, animal and nonfecal) on potential human health risk. Soller et al. (2014) evaluated mixtures of nonhuman and human fecal sources and reported corresponding enterococci densities equivalent to the EPA's recommended target illness rate. These studies concluded that RBTs for waters with predominantly nonhuman sources are likely to be higher than for waters impacted by predominantly human sources. Soller et al. (2010b) concluded that risks associated with exposure to recreational waters impacted by fresh gull, chicken, or pig feces can be substantially lower than waters impacted by human sources, when the water quality is anchored at 35 CFU per 100 mL enterococci or 126 CFU per 100 mL *E. coli*. Soller et al. (2014) concluded the predicted culturable enterococci densities that correspond to the RBT are substantially greater than the 2012 RWQC value for mixtures of human/nonpathogenic, human/gull, human/pig, and human/chicken inputs provided the human contribution was relatively low (Text Box G-1). Likewise, recreational waters containing a large fraction of culturable enterococci from nonhuman contamination sources may have reduced gastrointestinal illness risks to bathers compared to waters containing the same level of enterococci from human fecal sources.

Text Box G-1. Risk-Based Threshold

The output from the reverse QMRA is a FIB density that can be used as a geometric mean (GM) corresponding to the target illness rate and is called a Risk-Based Threshold (RBT).

Recent literature searches (Appendix A) have provided updated values for some QMRA parameters compared to those reported in Soller et al. (2010b, 2014). As part of this case study, the EPA conducted a sensitivity analysis comparing the new (base) analysis and Soller et al. (2014). See TSM Section 4.2.1 for a discussion of the sensitivity analysis.

By Step 4 (Section 3.4) of this TSM, users will have collected information to understand and confirm the fecal sources affecting the waterbody being characterized and have conducted a "forward" QMRA to understand the potential human health risk posed by the source loading to that waterbody. If the waterbody is shown to pose less risk compared to the EPA's target illness rates, then a "reverse" QMRA can be conducted to calculate a FIB density corresponding to a specific target illness rate. This appendix provides an example of how to adjust the 2012 RWQC recommendations to reflect risks from non-human sources, including using a "reverse" QMRA to calculate the GM. This case study example demonstrates an approach to document the reverse QMRA portion of this TSM. Examples of calculations for the STV and BAV are also provided. Users may find this case study example helpful for collating and documenting the type of information needed in preparing and submitting a WQS package including adjusted water quality criteria values to the EPA.

Methods

Each of the parameter values and model components used in this example reverse QMRA are indicated within tables in this section. Point estimates rather than stochastic distributions were used to describe all model parameters to ensure straightforward analytical computations in the reverse QMRA framework and for consistency with the form of the model output because the target illness rate was represented as a point estimate rather than a risk distribution. Information on model inputs is provided below.

Target Illness Rate

The case study example includes a target illness rate of 36 NGI per 1,000 recreators, which represents the upper illness rate included in the EPA's 2012 RWQC recommendations (U.S. EPA, 2012).

Ingestion Volume

Four different ingestion volume scenarios were modeled to capture variability across age groups and uncertainty in ingestion estimates. The ingestion parameters evaluated include the following point estimates:

- For the general population:
    - A median ingestion volume of 19 mL per swim event (Dufour et al., 2017).
    - A median ingestion volume of 16 mL per swim event (DeFlorio-Barker et al., 2017).
- For the 6–10 years old age group:
    - A median ingestion volume of 25 mL per swim event (Dufour et al., 2017).
    - A median ingestion volume of 40 mL per swim event (DeFlorio-Barker et al., 2017).

The general population (Dufour et al., 2017) provides the base analysis parameter for ingestion (19 mL per event), and the other three estimates are used in the sensitivity analyses. Section 2.2.2.2 describes these studies and the justification for the base analysis. Henceforth Dufour et al. (2017) is referred to as "Dufour" and DeFlorio-Barker et al. (2017) is referred to as "DFB."

The Python code (Appendix H) allows for one ingestion volume point estimate, so the base analysis and the three sensitivity analyses were run as separate iterations of the model.

Reference Pathogens in Human and Gull Contamination

The following reference pathogens were modeled for each source of contamination:

1. Human contamination: norovirus; as discussed in Section 2.1.1, norovirus was reported to account for over 70% of nonfoodborne illness with a viral etiology and approximately 63% of all nonfoodborne illness reported in the United States per year (Scallan et al., 2011a,b). Additionally, the norovirus dose-response relationship serves as an index for NGI caused by enteric viruses (Soller et al., 2010a).
2. Gull contamination: *Campylobacter jejuni* and *Salmonella*; as shown in Table 2-2 (in Section 2.1.1.1), these are the two pathogens that are most likely to occur in gull feces.

Dose-response Functions and Parameters

Table G-1 summarizes the dose-response functions and parameters used to estimate the probability of infection for each reference pathogen. The Beta Poisson model requires two parameters to define the probability of infection. The Beta Poisson model is typically approximated by: probability of infection equals $1 - (1 + \text{dose}/\beta)^{-\alpha}$. Both α and β are point estimates that provide the information that is unique for each pathogen. The Beta Poisson model with the confluent hypergeometric function can also use point estimates α and β, however in this case study a distribution was used for both of these parameters, called *w* and *z*.[42] The distributions are defined by the mean, variance, and covariance of the distribution as shown in Table G-1.

Table G-1. Dose-response functions and parameters (from TSM Table 2-5, Section 2.1.1.3.2).

Reference pathogen	Dose-response function	Parameter	Value/distribution
Norovirus	Beta Poisson model with the confluent hypergeometric function	*w**	mean = −0.608 variance = 1.79 covariance = −1.03
		*z**	mean = 0.194 variance = 2.54 covariance = −1.03
Campylobacter jejuni	Beta Poisson model with the confluent hypergeometric function	*w**	mean = −0.177 variance = 1.303 covariance = −0.041
		*z**	mean = 0.054 variance = 1.070 covariance = −0.041
Salmonella	Beta Poisson model approximation	α	0.3126
		β	2884

Note: * The w,z bivariate normal distributions can also be used to derive distributions for the α and β dose response parameters per the methods in Teunis et al. (2018) and Teunis et al. (2020).

Table G-2 summarizes the functions and parameters used to estimate the probability of illness given infection for each reference pathogen. The probability of illness given infection for *Salmonella* was modeled as a point estimate percentage. For norovirus and *Campylobacter,* the probability of illness given infection was based on the approach presented in Teunis et al. (2018) and Teunis et al. (2020), where this function is dependent on the dose and is called the hazard model function. The hazard model function can use either two point estimates or two distributions. The point estimates *r* and η can alternatively be represented by distributions *w* and *z*, which are incorporated into the model using the mean, variance, and covariance.[43]

[42] The choice of the symbols α, β, *w*, and *z* is consistent with the symbols used by Teunis et al. (2020) for these parameters.
[43] The choice of the symbols *r*, η, *w*, and *z* is consistent with the symbols used by Teunis et al. (2020) for these parameters.

Table G-2. Probability of illness functions and parameters (from TSM Table 2-6, Section 2.1.1.3.3).

Reference pathogen	Probability function	Parameter	Value/distribution
Norovirus	Hazard model function	*w**	mean = 1.74 variance = 5.55 covariance = −0.708
		*z**	mean = 1.82 variance = 4.64 covariance = −0.708
Campylobacter jejuni	Hazard model function	*w**	mean = −2.744 variance = 1.337 covariance = 0.010
		*z**	mean = -4.89×10^{-3} variance = 0.993 covariance = 0.010
Salmonella	Constant	c_pill midpoint of the range [0.17 to 0.4]	(0.17 + 0.40)/2.0

Note: * The *w*,*z* bivariate normal distributions can be used to derive distributions for the *r* and η hazard model parameters per the methods in Teunis et al. (2018) and Teunis et al. (2020).

Pathogen and FIB Levels in Contamination

Table G-3 shows the pathogen and enterococci levels in each type of fecal source. Each value is a point estimate that represents the midpoint of a reported range or a central tendency value for the parameter. The information from TSM Tables 2-2 and 2-7 were entered into the python code (Appendix H) as equations as shown in Table G-3. The value that the equation represents is also shown to illustrate the differences between sources in a transparent fashion. The level of norovirus in effluent was computed by dividing the level in human raw sewage by the average attenuation factor achieved in treatment plants.

Table G-3. Pathogen and enterococci levels in fecal source (from TSM Table 2-2 in Section 2.1.1.1 and Table 2-7 in Section 2.1.2.1).

Organism in fecal source (units)	Density in source	Density in source as shown in the python code
Enterococci in human raw (CFU/g)	7,943,282	10^((5.8+8.0)/2.0)
Enterococci in human effluent (CFU/L)	40	10^((0.5+2.7)/2.0)
Enterococci in gulls (CFU/g)	6,032,537	10^((0.5+2.7)/2.0)
Norovirus in human raw (genomes/g)	50,119	10^(4.7)
Norovirus in human effluent (genomes/L)	122	10^(4.7)/(10^((2.23+3)/2))
Salmonella in gulls (organisms/g)	446,684	10^((2.3+9.0)/2.0)
Campylobacter in gulls (organisms/g)	44,668	10^((3.3+6.0)/2.0)

Notes: The values in this table represent point estimates for the midpoint of the indicator and pathogen distributions. The value shown in the middle column is represented by an equation in the Appendix H python code (far right column). The equation in the python code uses the ranges shown in Table 2-2 and 2-7 (expressed as $\log_{10}$ exponents) divided by 2 to arrive at the midpoint of the range, except for norovirus in human raw, which is a central tendency point estimate.

Simulated Human Source Mix

The simulated human fecal source is a mixture of two sources: raw human and treated human effluent. The QMRA is anchored with two inputs from the EPA's 2012 RWQC, the target illness rate and the criteria magnitude (GM) for the FIB, in this case enterococci. The EPA's target illness rate of 36 NGI per 1,000 recreators is ultimately based on the EPA's epidemiological studies conducted in the 1970s (Dufour, 1984).[44] In order to have the level of enterococci at 35 CFU per 100 mL (the criteria magnitude) and the illness rate at 36 NGI per 1,000 recreators the human fecal source needs to have a blend of enterococci and pathogen levels that allow for this relationship. Neither human raw feces nor human effluent alone provides the correct ratio of pathogens to enterococci to result in the illness level of 36 NGI per 1,000 recreators at 35 enterococci CFU per 100 mL. Therefore a "simulated" mixture of human raw and effluent was computed to result in the correct anchoring values. The percentage of human source enterococci contributed by raw feces versus effluent was determined to be 1.11% and 98.89% respectively. This ratio of human source inputs allows 35 enterococci CFU per 100 mL to result in an illness level of 36 NGI per 1,000 recreators, thus anchoring the modeling to the epidemiological relationship. This simulated mixture was referred to as "idealized" by Schoen et al. (2011) and Soller et al. (2014), who first published this anchoring approach.

Pathogen Prevalence and Infectious Fraction Parameters

Table G-4 presents the prevalence and infectious fraction of the reference pathogens found in gull contamination. The minimum and maximum prevalence values from TSM Table 2-3 are converted into a point estimate that is the midpoint of the range. The percentages and the form of the values that are included in the python code (Appendix H) are both shown in Table G-4 for ease of understanding and transparency.

In human contamination, the prevalence and infectious fraction of norovirus were assumed to be 1 (100%). In other words, the prevalence of norovirus in the human source is 100% and the fraction of norovirus in the human source that is infectious is 100%.

Table G-4. Prevalence and infectious fraction of reference pathogens in gull contamination (from TSM Tables 2-3 and 2-4 in Section 2.1.1.1).

Reference pathogen	Prevalence percentage	Prevalence as shown in python code*	Infectious percentage	Infectious fraction as shown in python code**
Campylobacter jejuni	77%	(0.54+1)/2	16.7%	0.167
Salmonella	87.5%	(0.75+1)/2	16.7%	0.167

Notes:

* The prevalence values in this table represent point estimates for the midpoint of the minimum and maximum pathogen prevalence estimates.

** The fraction of human infectious strains represents a qualitative estimate of "low" that was converted to 0.167 as the midpoint of 0 and 0.33 per Soller et al. (2010b).

[44] As described in the 2012 RWQC, EPA translated the level of HCGI that corresponded with 35 enterococci CFU per 100 mL in Dufour (1984) to the level of NGI from the EPA NEEAR studies that corresponded with 35 enterococci CFU per 100 mL (Wymer et al., 2013).

Proportion of FIB from Human Fecal Sources

The percentage of enterococci contributed by human fecal sources was calculated at multiple levels to reflect a range of scenarios, including 10%, 20%, 25%, 30%, 33%, 50%, 60%, 67%, 75%, 90%, and 100%. The Python Code in Appendix H includes these fecal source apportionment scenarios in a single run.

Initial FIB Level Estimates

Due to the iterative nature of the reverse QMRA process as applied in Step 4 of this TSM, an initial value for the FIB level was needed to initiate the modeling. An FIB level of 50 CFU/100 mL (in the code 500 CFU/L) was provided as an initial estimate of the threshold FIB level corresponding to the target risk level for each mixture type. This value was refined by computer code through numerical iteration to derive the specific threshold FIB level applicable for each mixture type and at each simulated human source contribution level as described in the analytical approach.

Analytical Approach

This section presents the analytical approach for:

- The reverse QMRA approach used to derive the adjusted FIB GM.
- The statistical approach used to derive the STV and BAV criteria based on the QMRA results.

The analytical approach references the TSM to which users may refer for detailed explanation of the equations and terminology.

Reverse QMRA Approach

A preliminary estimate for the enterococci RBT for the different example scenarios was applied to compute the density of each reference pathogen attributable to each source as described in TSM Equations 3A and 3B. This initial RBT estimate was later refined by numerical iteration during the reverse QMRA as described below. For definition of parameters see Section 2.2.2.4.5.

$$C_{rp}^{NHS} = p_{FIB}^{NHS} \times C_{FIB} \times \frac{R_{rp}^{NHS}}{R_{FIB}^{NHS}} \quad \text{[Eq. 3A]}$$

$$C_{rp}^{HS} = p_{FIB}^{HS} \times C_{FIB} \times \frac{R_{rp}^{HS}}{R_{FIB}^{HS}} \quad \text{[Eq. 3B]}$$

As the human source was a mixture of raw and secondary treated and disinfected WWTP effluent, the ratio of pathogens to FIB in the human source in TSM Equation 3B was computed as follows:

$$\frac{R_{rp}^{HS}}{R_{FIB}^{HS}} = 0.9889\frac{R_{rp}^{raw}}{R_{FIB}^{raw}} + 0.0111\frac{R_{rp}^{WWTP}}{R_{FIB}^{WWTP}} \quad \text{[Eq. 3C]}$$

Note that the constants in TSM Equation 3C were derived by anchoring a probability of illness of 36 per 1,000[45] to an enterococci level of 35 CFU per 100 mL for an ingestion volume of 0.019 L (i.e., anchoring to the EPA's recommended target illness rate and corresponding water quality value).

[45] The recommended health goal associated with the national 2012 RWQC is 32 or 36 NGI illnesses per 1,000 primary contact recreation events. These two values are not significantly different.

The dose of each reference pathogen attributable to each source was computed per TSM Equations 3D and 3E.

$$\mu_{rp}^{NHS} = V \times C_{rp}^{NHS} \times p_{rp}^{NHS} \times I_{rp}^{NHS} \quad \text{[Eq. 3D]}$$

$$\mu_{rp}^{HS} = V \times C_{rp}^{HS} \quad \text{[Eq. 3E]}$$

The probability of infection from exposure to each reference pathogen derived from each source was computed per TSM Equations 3F and 3G.

$$P_{inf}^{rp,NHS} = f_{d-r}^{rp}\,(\mu_{rp}^{NHS}) \quad \text{[Eq. 3F]}$$

$$P_{inf}^{rp,HS} = f_{d-r}^{rp}\,(\mu_{rp}^{HS}) \quad \text{[Eq. 3G]}$$

The probability of illness from exposure to each reference pathogen derived from each source was computed per TSM Equations 3H and 3I.

$$P_{ill}^{rp,NHS} = p_{ill|inf}^{rp} \times P_{inf}^{rp,NHS} \quad \text{[Eq. 3H]}$$

$$P_{ill}^{rp,HS} = p_{ill|inf}^{rp} \times P_{inf}^{rp,HS} \quad \text{[Eq. 3I]}$$

The probability of illness from exposure to each source, accounting for the effect of all reference pathogens within that source was computed per TSM Equations 3J and 3K.

$$P_{ill}^{NHS} = 1 - \prod_{rp}(1 - P_{ill}^{rp,NHS}) \quad \text{[Eq. 3J]}$$

$$P_{ill}^{HS} = 1 - \prod_{rp}(1 - P_{ill}^{rp,HS}) \quad \text{[Eq. 3K]}$$

The total probability of illness accounting for exposure to both the human and nonhuman sources was computed using TSM Equation 3L.

$$P_{ill} = 1 - \left(1 - P_{ill}^{NHS}\right) \times (1 - P_{ill}^{HS}) \quad \text{[Eq. 3L]}$$

As the computed value of the total probability of illness in Equation 3L differs from the target probability of illness, the initial estimate for the RBT in the waterbody was iteratively adjusted by the computer program until the computed value agreed with the target value of probability of illness. The FIB RBT in the final iteration of this procedure is the waterbody FIB density level that corresponds to the target probability of illness for the specified contamination mixture.

The approach for this analysis has been also implemented in computer code, which is included in Appendix H (reverse QMRA example in Python code). This code example may be modified for specific applications and may be further adapted to work in batch mode to run sensitivity analyses. The Python code was run four separate times to account for each of the ingestion scenarios. The fecal source apportionment scenarios are built into the code, so are run as a batch each time the code is run. The user defines a location for an output file, which presents the results in a table with 5 rows (a header and each of the four sources modeled in the code) and 12 columns (the row labels, and 11 apportionment scenarios).

Derivation of STV and BAV

The example STVs and BAVs were derived based on the magnitude of the site-specific criteria obtained from the reverse QMRA approach. The statistical formulas for deriving the STV and BAV are provided in TSM Section 3.4.2. In this case study the standard deviation (SD) utilized by the EPA in the 2012 RWQC was applied (0.44 logSD for enterococci).[46]

The approach for this analysis has been also implemented in computer code, which is included in Appendix I (STV and BAV calculation example in R code). This code example may be modified for specific applications and may be further adapted to work in batch mode to run sensitivity analyses. Results

RBT Threshold—GM

Figure G-1 shows the results of the base analysis, which includes the median ingestion volume of 19 mL for varying gull/human fecal mixtures. The nonpathogenic/human mixture results are not shown in Figures G-1, G-2, and G-3 because they are so similar to the gull/human results that the icons would overlap.

Each of the points on Figure G-1 represent an enterococci density consistent with a 36 NGI per 1,000 recreators target illness rate. The dotted horizontal line represents the EPA's recommended enterococci value of 35 CFU enterococci per 100 mL. The point at 100% human source is, by definition, anchored at 35 CFU enterococci per 100 mL because the epidemiological-based health relationship was characterized at beaches affected by human fecal sources (Cabelli, 1983; Dufour, 1984). At 33% human source (and 66% gull source) there is about a half log difference between the RBT and the RWQC. At 10% human source (and 90% gull source) there is a log difference between the RBT and the RWQC.

[46] As noted in Section 3.4.2, if estimates of the SD of the indicator levels for the evaluated waterbody are not available, the SDs utilized by EPA in the 2012 RWQC can be applied (0.44 logSD for enterococci or 0.40 logSD for *E. coli*).

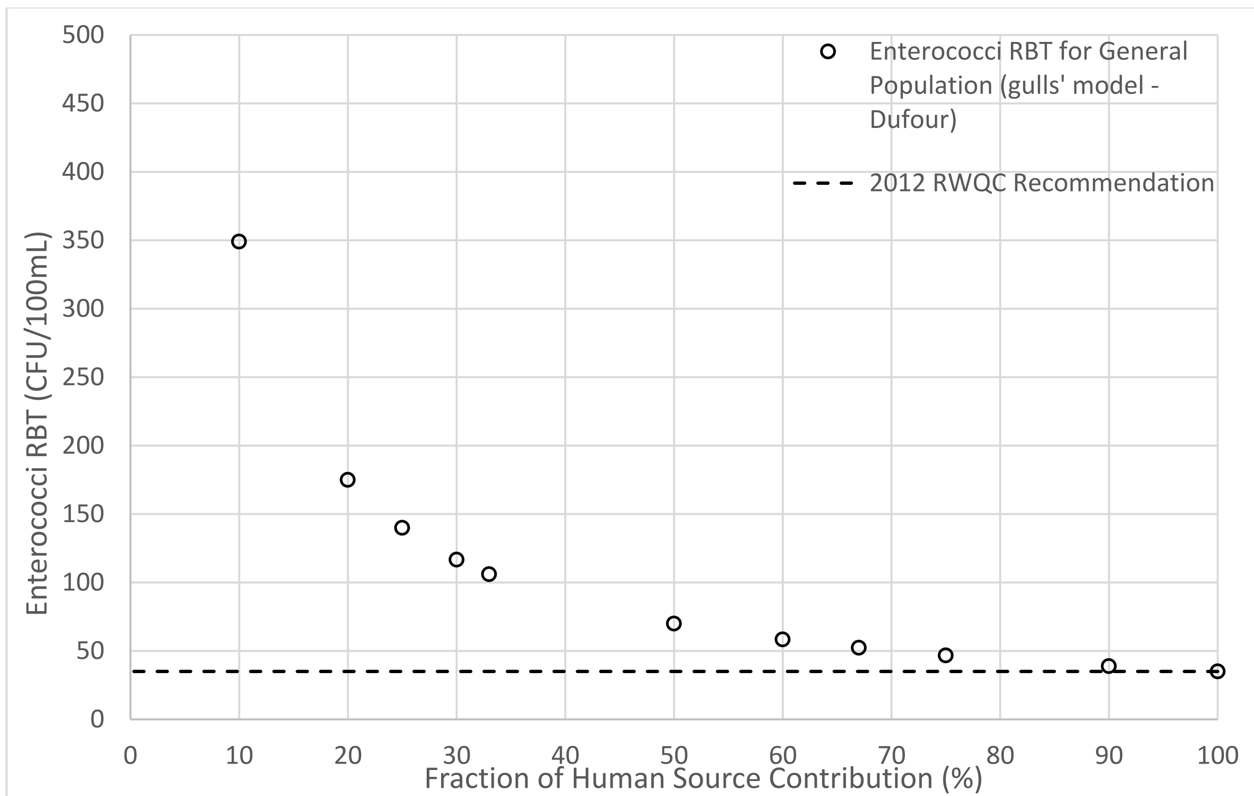


Figure G-1. Base analysis results—enterococci RBT for waters impacted by human and gull fecal mixtures.

Figure G-2 compares the RBTs using the general population ingestion volumes from Dufour and DFB. The lower ingestion volume from the DFB study (16 mL/event) versus Dufour (19 mL/event) results in higher RBTs than the base analysis. The incidental ingestion volume is the primary exposure pathway considered within the scope of the QMRA.

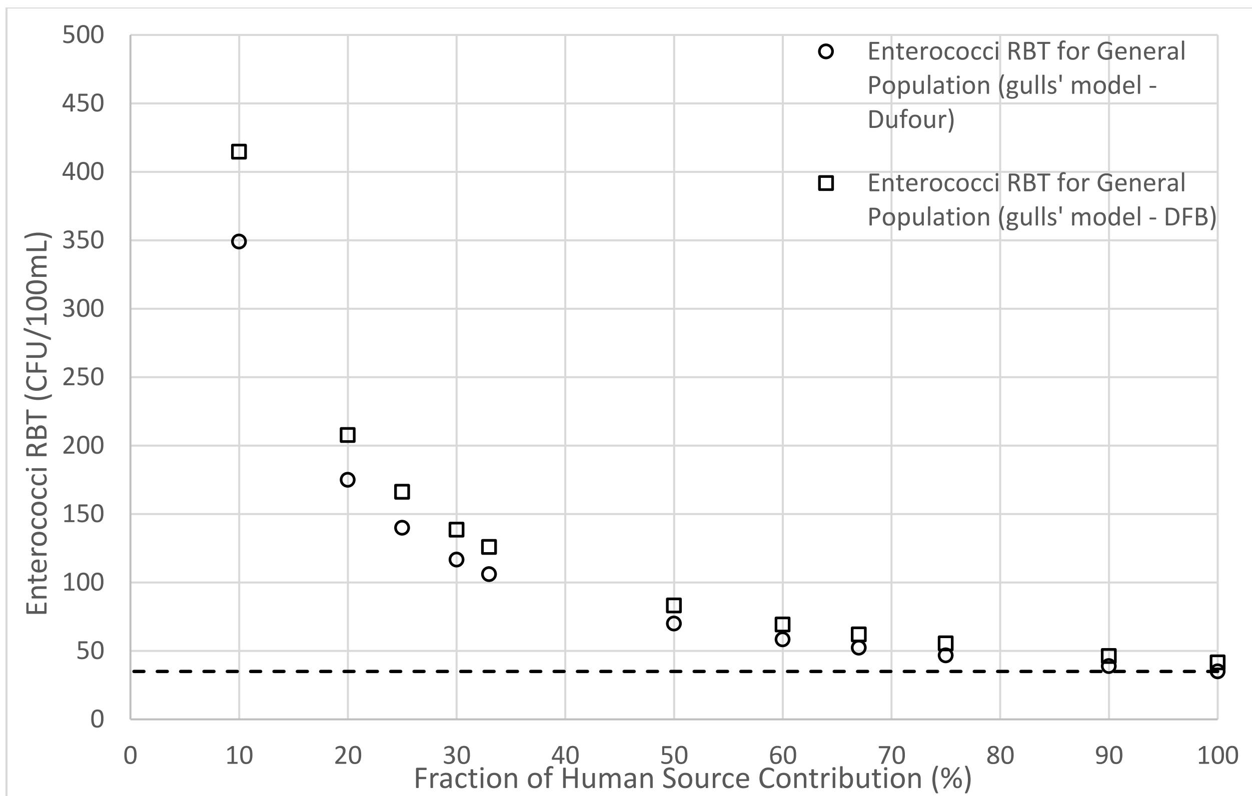


Figure G-2. Enterococci RBTs corresponding to 36 NGI per 1,000 recreators for the general population using estimates of incidental ingestion from Dufour et al. (2017) and DeFlorio-Barker et al. (2017).

Figure G-3 compares the RBTs for the general population and the 6 to 10 years old age group using the median ingestion volumes from Dufour. The higher ingestion volumes for children ages 6 to 10 years old (25 mL/event) results in RBTs that are lower than the base analysis. It is worth noting that at 100% human contribution, the enterococci RBT for children 6–10 years old is lower than the RWQC recommendation because the RWQC is benchmarked to the general population health relationship.

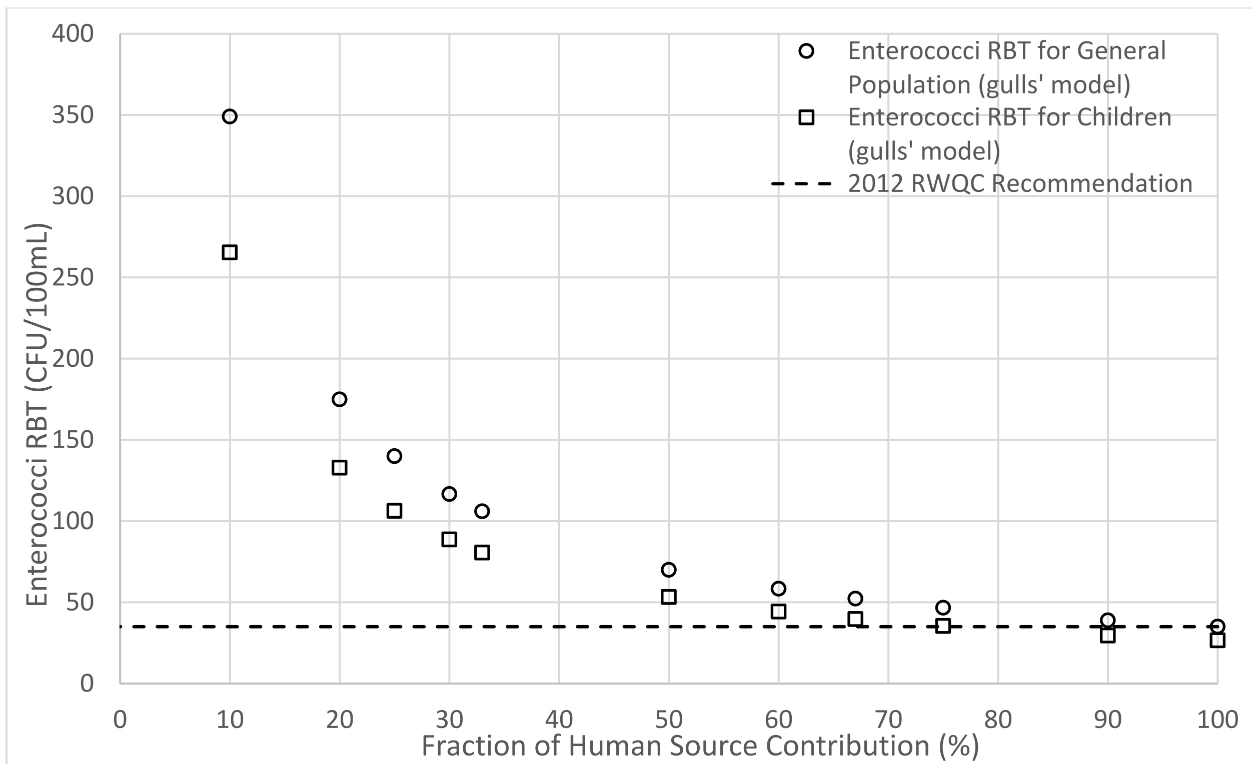


Figure G-3. Enterococci RBTs corresponding to 36 NGI per 1,000 recreators for the general population and children ages 6 to 10 years.

Table G-5 presents the enterococci RBTs for gull/human fecal source mixtures at different fractions of human fecal contributions. All four ingestion scenarios are shown (base analysis and three sensitivity analyses). The model was anchored at 35 CFU enterococci per 100 mL with the general population Dufour ingestion scenario (base analysis). Similarly, Table G-6 presents the enterococci RBT for nonpathogenic/human source mixtures. The RBTs for gull/human and nonpathogenic/human mixtures are the same except for two values at the 10% level where the CFU differ by 1.

Table G-5. RBTs (enterococci CFU/100 mL) for waters impacted by human and gull contamination at 36 NGI per 1,000 recreators.

Ingestion scenario	Percentage of human source FIB contribution		
	≤ 10%	≤ 25%	≤ 33%
General population (Dufour)	349	140	106
General population (DFB)	415	166	126
6 to 10 (Dufour)	265	106	81
6 to 10 (DFB)	166	66	50

Table G-6. RBTs (enterococci CFU/100 mL) for waters impacted by human and nonpathogenic contamination at 36 NGI per 1,000 recreators.

Ingestion scenario	Percentage of human source FIB contribution		
	≤ 10%	≤ 25%	≤ 33%
General population (Dufour)	350	140	106
General population (DFB)	415	166	126
6 to 10 (Dufour)	266	106	81
6 to 10 (DFB)	166	66	50

STV and BAV Calculations

The values in Table G-5 were used to calculate the corresponding STVs (Table G-7) and BAVs (Table G-9) for gull/human sources. The values in Table G-6 were used to calculate the corresponding STVs (Table G-8) and BAVs (Table G-10) for nonpathogenic/human sources. Example R code for calculating the STV and BAV is available in TSM Appendix I.

Table G-7. STVs (enterococci CFU/100 mL) for waters impacted by human and gull contamination.

Ingestion scenario	Percentage of human source FIB contribution		
	≤ 10%	≤ 25%	≤ 33%
General population (Dufour)	1279	512	388
General population (DFB)	1519	609	461
6 to 10 (Dufour)	972	389	295
6 to 10 (DFB)	608	243	184

Table G-8. STVs (enterococci CFU/100 mL) for waters impacted by human and nonpathogenic contamination.

Ingestion scenario	Percentage of human source FIB contribution		
	≤ 10%	≤ 25%	≤ 33%
General population (Dufour)	1282	513	388
General population (DFB)	1522	609	461
6 to 10 (Dufour)	974	390	295
6 to 10 (DFB)	609	244	184

Table G-9. BAVs (enterococci CFU/100 mL) for waters impacted by human and gull contamination.

Ingestion scenario	Percentage of human source FIB contribution		
	≤ 10%	≤ 25%	≤ 33%
General population (Dufour)	691	277	210
General population (DFB)	821	329	249
6 to 10 (Dufour)	526	211	160
6 to 10 (DFB)	328	132	100

Table G-10. BAVs (enterococci CFU/100 mL) for waters impacted by human and nonpathogenic contamination.

Ingestion scenario	Percentage of human source FIB contribution		
	≤ 10%	≤ 25%	≤ 33%
General population (Dufour)	693	277	210
General population (DFB)	823	329	249
6 to 10 (Dufour)	527	211	160
6 to 10 (DFB)	329	132	100

Discussion

The enterococci RBTs derived above reflect scenarios where gull feces (or nonpathogenic FIB sources) are the predominant source of enterococci in the waterbody. Varying levels of human to gull source contributions were modeled to provide context. The enterococci RBTs provide a GM threshold for the hypothetical site that is consistent with the EPA's recommended target illness rate. For example, if the waterbody was determined to have predominantly fecal loading from gull feces with an approximate 25% human fecal contribution, the reverse QMRA output GM value of 140 enterococci CFU/100 mL (Table G-5) could be chosen as the adjusted water quality criteria value. Table G-5 only shows human fecal sources below 33% because waters with low human inputs are candidates for alternative RBTs.

Figure G-1 shows the enterococci density corresponding to the target illness rate for the base analysis for 11 different fecal apportionment scenarios. The model anchors the 100% human source at 35 enterococci CFU per 100 mL. The fecal apportionment scenarios with less human fecal source and more gull fecal source have higher corresponding enterococci densities corresponding to the target illness rate. For example, at ≤ 10% human fecal sources the corresponding enterococci density RBT is 349 enterococci CFU per 100 mL (Table G-5).

Figure G-2 shows the base scenario compared to the ingestion volume for the general population calculated in DFB. The lower ingestion rate from DFB results in higher enterococci densities corresponding to the target illness rate for each of the fecal apportionment scenarios. The EPA chose the general population data from Dufour for the base analysis because it represents empirically measured ingestion data, whereas the ingestion results from DFB are modeled from estimated time spent in the water for NEEAR study participants combined with the Dufour ingestion data.

Figure G-3 shows the base scenario compared to the ingestion volume for children ages 6 to 10 years old from Dufour. The higher ingestion rate for children 6 to 10 years old results in lower enterococci

densities corresponding to the target illness rate for each of the fecal apportionment scenarios. At 100% human source the base scenario is anchored at 35 enterococci CFU per 100 mL and for children 6 to 10 years old the 100% human source is below the anchor line (Figure G-3).

For the base scenario a BAV value of 277 and an STV value of 513 enterococci CFU/100 mL could be chosen consistent with the GM (Tables G-9 and G-7). The EPA expects users to apply the frequency consistent with the unadjusted WQS and determine the duration as part of any alternative WQS submitted to the EPA.

The results of this case study confirm earlier analyses, including Soller et al. (2014) and Soller et al. (2010b), that found sea gulls represent a very low risk fecal source when they are the predominant source of contamination. The case study illustrates how GM RBTs, STVs, and BAVs, may be derived, and the sensitivity of the QMRA output to alternative assumptions for mixtures with degrees of nonhuman contamination. In each case, the RBTs are considerably higher than if only human source contamination were present. The almost identical results for human and nonpathogenic mixtures, compared to human and sea gull contamination mixtures, is further evidence that sea gull contamination contributes a very low level of risk.

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Appendix H: Python Code for Appendix G

This appendix provides the annotated code for the Appendix G Gull Case Study. The code estimates the FIB (in this case enterococci) risk-based thresholds (RBTs) corresponding to a target illness rate for the gull/human fecal mixtures and nonpathogenic/human fecal mixtures. Although the Appendix G Gull Case Study does not include swine or chicken sources, the code also includes swine (pig)/human and chicken/human fecal mixtures. It is recommended that this code be utilized by experienced programmers after a careful reading of its syntax and structure with reference to the appropriate equations described in the TSM. This code should not be considered a tool. In this example code all the references to FIB refer to enterococci, however, the code can be adapted for other FIB such as *E. coli*. Note that the code outputs RBTs in units of CFU/100 mL. If the simulated human fecal source needs to be adjusted because a different target illness rate or FIB is used, this code could be adapted. Example constants for the simulated "idealized" human mixture of effluent to raw are in Table H-1. A key for the annotated code is presented in Table H-2 and the definitions and sources for variables for the Appendix G Gull Case study is presented in Table H-3.

Table H-1. Constants for simulated human mixtures for other target illness rates and FIB.

2012 RWQC target illness rate	FIB density	ratio_hum_raw	ratio_hum_wwtp
36 NGI/1,000 recreators	35 CFU/100 mL[a] enterococci (culture-based)	0.9889	0.0111
32 NGI/1,000 recreators	30 CFU/100 mL[b] enterococci (culture-based)	0.9882	0.0118
36 NGI/1,000 recreators	126 CFU/100 mL[c] *E. coli* (culture-based)	0.9869	0.0131
32 NGI/1,000 recreators	100 CFU/100 mL[c] *E. coli* (culture-based)	0.9845	0.0155

Notes:

a. This is the mixture shown in the code below and corresponds to the scenario described in Appendix G.

b. In this example code, 100% human source is benchmarked to 36 NGI per 1,000 recreators and 35 CFU enterococci per 100 mL. If 32 NGI per 1,000 recreators is chosen as the target illness rate, the associated enterococci is 30 CFU per 100 mL. The two values for ratio_hum_raw and ratio_hum_wwtp would need to be replaced by the values shown in the table.

c. If *E. coli* is used, the two values for ratio_hum_raw and ratio_hum_wwtp would need to be replaced by the values shown in the table corresponding to the target illness rate chosen. In addition, all the FIB densities for each source would also need to be changed to correspond with *E. coli* densities.

Table H-2. Key for annotated code.

Key
BOLD—Actual code for python (software available at http://www.python.org/getit/)
PLAIN—Comments for guidance

Table H-3. Appendix G Gull Case Study: definitions and sources for variables in equations.

In-text variable	Code variable X represents the pathogen of interest	Definition
$C_{rp}^{NHS}, C_{rp}^{HS}$	No explicit variable defined. Computation embedded within the dose function for program efficiency.	Estimated waterbody density of reference pathogen (number of pathogens or genomes/L) in nonhuman and human sources.
p_{FIB}^{NHS}	pcfu	Proportion of FIB density attributable to the nonhuman source NHS (unitless; evaluated here as a vector assuming multiple values)
C_{FIB}	fib_dens	Initial estimate for FIB RBT (CFU/L) corresponding to the target probability of illness for each proportion.
R_{rp}^{NHS}	xx_dens['PathogenName']	Density of reference pathogens (number of pathogens or genomes/L or number of pathogens or genomes/g) in the nonhuman source NHS
R_{FIB}^{NHS}	xx_dens['ent']	Density of FIB (CFU/L or CFU/g) in the nonhuman source NHS
R_{rp}^{raw}	hum_raw_dens['nor']	Density of each reference pathogen (number of pathogens or genomes/g) in raw human contamination;
R_{rp}^{WWTP}	hum_wwtp_dens['nor']	Density of each reference pathogen (number of pathogens or genomes/L) in WWTP-treated human contamination;
R_{FIB}^{raw}	hum_raw_dens['ent']	Density of FIB (CFU/g) in raw human contamination
R_{FIB}^{WWTP}	hum_wwtp_dens['ent']	Density of FIB (CFU/L) in WWTP-treated human contamination
$\mu_{rp}^{NHS}, \mu_{rp}^{HS}$	dose['fib_dens','PathogenName']	Estimated dose ingested of reference pathogen from nonhuman and human sources respectively (number of pathogens). Common implementation in dose function.
V	vol_ing	Volume of water ingested (L)
p_{rp}^{NHS}	sw_frinf['PathogenName']	Fraction of human infectious pathogenic strains (unitless)
I_{rp}^{NHS}	sw_prev['PathogenName']	Prevalence of infection in the nonhuman source (unitless)
$p_{ill\|inf}^{rp}$	drp_X['c_pill']	Proportion of infections resulting in illness (unitless)
f_{d-r}^{rp}	drf_bp,drf_exp,drf_hyp. Dose response parameters for these functions are found in drp_X.	Dose-response functions for various pathogens (unitless)
$P_{ill}^{rp,NHS}, P_{ill}^{rp,HS}$	pill_X. (No explicit superscripting was required for NHS and HS because the pathogens found in the two sources did not overlap.)	Probability of illness from a specific reference pathogen rp originating from the nonhuman source NHS and human source HS, respectively (unitless)
$P_{ill}^{NHS}, P_{ill}^{HS}$	Directly implemented in tpill function below.	Estimated probability of illness from the nonhuman source NHS and human source HS, respectively (unitless)
P_{ill}	tpill	Estimated total probability of illness accounting for exposure to both the nonhuman and human sources of contamination (unitless)

```
#!/usr/local/bin/python

#########################################################################################
### Example code for implementing a "reverse" QMRA. Determines FIB density
### corresponding to specified level of risk for contamination mixtures deriving
### from human and alternative animal (swine, chicken, gull, non-pathogenic) sources.
### Uses the Teunis 2020 and 2018 Bayesian dose response function for norovirus and camp. jejuni
#########################################################################################

from numpy import *          ### Imports Python NumPy module
from scipy.optimize import fsolve  ### Imports necessary sub-module from Python SciPy module
from scipy.special import hyp1f1  ### Imports necessary sub-module from Python SciPy module

random.seed(1) ### Ensures numerical replicability
print ("REVERSE QMRA EXAMPLE FOR ANIMAL AND HUMAN MIXTURES USING THE BAYESIAN TEUNIS DRFs FOR NORO
and CJ ") ## Prints output message
print("")
print( "FINDS FIB DENSITY CORRESPONDING TO SPECIFIED LEVEL OF RISK") ## Prints output message
print("")

risk=0.036   ### Target illness rate from the 2012 RWQC, specified as a probability.
    ### In this case, the chosen level of risk corresponds to 36 illnesses per 1000 swim events (unitless).
vol_ing=0.019  ### Volume of water ingested during the recreational event, in L. General population median value from
Dufour et al. 2017.

### The following lines create arrays of FIB and pathogen densities found in swine, chicken and gull contamination
### based on the midpoint of the range of values in TSM Section 2 Tables 2-2 and 2-7.
### Array of FIB (cfu/g) and pathogen (organisms/g) densities found in swine contamination
sw_dens={'ent':10**((5.3+7.2)/2.0),'cj':10**((2.0+5.7)/2.0),'g':10**((0+6.8)/2.0),'s':10**((2.8+4.9)/2.0),'cr':10**((1.7+3.6)
/2.0),'c57':10**((0.0+7.0)/2.0)}
### Array of FIB (cfu/g) and pathogen densities (organisms/g) found in chicken contamination
ch_dens={'ent':10**((5.0+7.0)/2.0),'cj':10**((2.8+6.5)/2.0),'g':0,'s':10**((-1.0+4.5)/2.0),'cr':0,'c57':0}
### Array of FIB (cfu/g) and pathogen (organisms/g) densities found in gull contamination
gu_dens={'ent':10**((4.114+9.447)/2.0),'cj':10**((3.3+6.0)/2.0),'g':0,'s':10**((2.3+9.0)/2.0),'cr':0,'c57':0}

### Array of FIB (cfu/L) and pathogen (organisms/L) densities found in non-pathogenic contamination.
### Artificial density value used for enterococci density to avoid 0/0 error when calculating path:fib ratio.
np_dens={'ent':0.1,'cj':0,'g':0,'s':0,'cr':0,'c57':0}
### Nested dictionary of animal source pathogen and FIB densities.
dens_source={'swine':sw_dens,'chicken':ch_dens,'gulls': gu_dens,'non-path':np_dens}

### The following lines create arrays of pathogen prevalence rates for swine, chicken and gull contamination
### based on the midpoint of the range of values in TSM Section 2 Table 2-3.
### Array of pathogen prevalence rates for swine contamination (unitless)
sw_prev={'cj':(0.46+0.98)/2,'g':(0.033+0.18)/2.0,'s':(0.079+0.15)/2.0,'cr':(0+0.45)/2.0,'c57':(0.001+0.12)/2.0}
### Array of pathogen prevalence rates for chicken contamination (unitless)
ch_prev={'cj':(0.57+0.69)/2,'g':0,'s':(0.0+0.95)/2.0,'cr':(0.06+0.27)/2.0,'c57':0}
### Array of pathogen prevalence rates for gull contamination (unitless)
gu_prev={'cj':(54+100)/2/100,'g':0,'s':(75+100)/2/100,'cr':0,'c57':0}
### Array of pathogen prevalence rates for non-pathogenic contamination (unitless)
np_prev={'cj':0.0,'g':0,'s':0.0,'cr':0,'c57':0}
### Nested dictionary of pathogen prevalence rates.
prev_source={'swine':sw_prev,'chicken':ch_prev,'gulls':gu_prev,'non-path':np_prev}
```

```
### The following lines create arrays of pathogen infectious fraction estimates for swine, chicken and
### gull contamination based on TSM Section 2 Table 2-4.
### Array of pathogen infectious fraction estimates for swine contamination (unitless).
sw_frinf={'cj':0.833,'g':0.833,'s':0.5,'cr':0.167,'c57':0.833}
### Array of pathogen infectious fraction estimates for chicken contamination (unitless).
ch_frinf={'cj':0.5,'g':0,'s':0.5,'cr':0.167,'c57':0}
### Array of pathogen infectious fraction estimates for gull contamination (unitless).
gu_frinf={'cj':0.167,'g':0,'s':0.167,'cr':0,'c57':0}
### Array of pathogen infectious fraction estimates for non-pathogenic contamination (unitless)
np_frinf={'cj':0.0,'g':0,'s':0.0,'cr':0,'c57':0}
### Nested dictionary of infectious fraction rates.
frinf_source={'swine':sw_frinf,'chicken':ch_frinf,'gulls':gu_frinf,'non-path':np_frinf}

### The following line creates an array of FIB and pathogen densities found in raw human contamination
### based on the midpoint of the range of values in TSM Section 2 Tables 2-2 and 2-7.
### Array of FIB (cfu/L) and pathogen (organisms/L) densities found in raw human contamination
hum_raw_dens={'ent':10**((5.8+8.0)/2.0),'nor':10**(4.7)}
### Norovirus attenuation level achieved in WWTP plants based on the midpoint of the range of values in Soller, et al.
(2010b).
wwtp_atten=10**((2.23+3)/2)

### The following line creates an array of FIB and pathogen densities found in WWTP-treated human contamination based
### on the midpoint of the range of values in TSM Section 2 Table 2-3 and the attenuation value specified in the line above.
### Array of FIB (cfu/L) and pathogen (organisms/L) densities found in WWTP-treated human contamination
hum_wwtp_dens={'ent':10**((0.5+2.7)/2.0),'nor':hum_raw_dens['nor']/wwtp_atten}
### Ratio of pathogen to FIB levels in raw human contamination
ratio_hum_raw=hum_raw_dens['nor']/hum_raw_dens['ent']
### Ratio of pathogen to FIB levels in WWTP-treated human contamination
ratio_hum_wwtp=hum_wwtp_dens['nor']/hum_wwtp_dens['ent']

### Ratio of pathogen to enterococci level in mixture of raw and WWTPtreated human contamination.
### The fractions of raw and WWTP
### contamination in the mixture were determined such that an ingestion volume of 0.019 L results in an illness rate
### of 36/1000 and an enterococci level of 35 cfu/100mL.
### This implementation is based on Equation 3C and represents the simulated human source
ratio_dens_hmix=0.9889*ratio_hum_raw+0.0111*ratio_hum_wwtp

### Array specifies alternative levels of non-human source contribution of total FIB level in surface water. For
### instance, 0 corresponds to 100% human contribution; 0.1 corresponds to 10% non-human source and 90% human
### contribution, etc.
pcfu=array([0,0.1,0.25,0.33,0.4,0.5,0.67,0.7,0.75,0.8,0.9])
### Array contains an initial estimate of FIB levels in CFU/L corresponding to specified risk for the different
### percentages of the mixture defined in the line above.
fib_dens=array([500,500,500,500,500,500,500,500,500,500,500])
### The function "dose" defined below computes the dose of the specified pathogen ingested during a swim event by
### estimating the % of FIB from the source in which that pathogen is found, multiplying it by the ratio of pathogens
### per FIB in that source, and then multiplying that by the volume of water ingested.
### This implementation is based on Equations 3A, 3B, 3D and 3E.
def dose(fib_dens,PathogenName,AnimalSource):
  if PathogenName=='nor':
    result = (1-pcfu)*fib_dens*ratio_dens_hmix*vol_ing
  else:
    result =
pcfu*fib_dens*dens_source[AnimalSource][PathogenName]/dens_source[AnimalSource]['ent']*prev_source[AnimalSou
rce][PathogenName]*frinf_source[AnimalSource][PathogenName]*vol_ing
  return result
```

```
### Beta Poisson Dose Response Function based on TSM Section 2 Table 2-5.
def drf_bp(a,b,c_pill,d):
  return (1-(1+d/b)**-a)*c_pill

### Exponential Dose Response Function based on TSM Section 2 Table 2-5.
def drf_exp (r,c_pill,d):
  return(1-exp(-r*d))*c_pill

##################################################################################################
## The functions below implement the Teunis 2018 and Teunis 2020
### dose response functions with parameters jointly estimated by the Bayesian posterior approach
##################################################################################################
#

n_samp=10000 # number of samples to draw from posterior distribution

### This function jointly samples w and z (transformed parameters) from posterior distribution expressed as bivariate
normal
### inputs are vector of means and the variance covariance matrix
def sample_w_z(means, vcov,n):
   return(random.multivariate_normal(means,vcov,n).T)

### This function computes probability of infection based on w, z parameter distribution using the confluent
hypergeometric function
### Inputs are joint w,z distribution and dose
def pinf_t20(inf_dist,d):
   w=inf_dist[0]; z=inf_dist[1]
   u=exp(w)/(1+exp(w)); v=exp(z)
   alpha=v*u; beta=v*(1-u)
   p=1-hyp1f1(alpha,(alpha+beta),-d)
   p=clip(p, 0, 1)
   return(p)

### This function computes conditional probability of illness based on w, z parameter distribution
### Inputs are joint w,z distribution and dose
def cpill_t20(ill_dist,d):
   w=ill_dist[0]; z=ill_dist[1]
   u=exp(w)/(1+exp(w)); v=exp(z)
   r=v*u; eta=v*(1-u)
   p=1-(1+d/eta)**-r
   p=clip(p, 0, 1)
   return(p)

### This function computes the median probability of illness combining probability of infection and conditional probability
of illness given infection
### Inputs are joint w,z distribution for both infection and illness, and dose

def drf_hyp_t20(inf_dist,ill_dist,dose):
   pill_vector=[]
   for d in dose:
      pinf=pinf_t20(inf_dist,d)
      cpill=cpill_t20(ill_dist,d)
      pill=pinf*cpill
      pill_vector.append(median(pill))
   return(array(pill_vector))
```

```
##########################################################################################################

### Dose Response Parameters for salmonella based on Table 2-5
drp_s={'a':0.3126,'b':2884,'c_pill':(0.17+0.40)/2.0}
### Dose Response Parameters for giardia based on
drp_g={'r':0.0199,'c_pill':(0.2+0.7)/2.0}
### Dose Response Parameters for cryptosporidium based on Table 2-5
drp_cr={'r':0.09,'c_pill':0.5}
### Dose Response Parameters for e-coli O157:H7 based on Table 2-5
drp_c57={'a':0.248,'b':48.8,'c_pill':(0.2+0.6)/2.0}
### Dose Response Parameters for norovirus based on Table 2-5
drp_nor={'inf_means':[-0.608, 0.194],'inf_vcov':[[1.79, -1.03], [-1.03, 2.54]],'ill_means': [1.74, 1.82],'ill_vcov':[[5.55, -
0.708], [-0.708, 4.64]]}
### Dose Response Parameters for campylobacter jejuni based on Table 2-5
drp_cj={'inf_means':[-0.177,0.054],'inf_vcov':[[ 1.303,-0.041], [-0.041,1.070]],'ill_means': [-2.744,-4.89E-
3],'ill_vcov':[[1.337, 0.010], [0.010, 0.993]]}

############################################################
### Sample from normalized parameter posterior distributions
### for DRFs based on Teunis 2018 and 2020
###(Generate w,z distributions for infection and illness)
############################################################

nor_inf_dist=sample_w_z(drp_nor['inf_means'], drp_nor['inf_vcov'],n_samp)
nor_ill_dist=sample_w_z(drp_nor['ill_means'], drp_nor['ill_vcov'],n_samp)
cj_inf_dist=sample_w_z(drp_cj['inf_means'], drp_cj['inf_vcov'],n_samp)
cj_ill_dist=sample_w_z(drp_cj['ill_means'], drp_cj['ill_vcov'],n_samp)

### The following functions are implementations of the generic representation in Equation 3F, 3G, 3H, and 3I

### The function pill_cj computes the probability of illness from exposure to campylobacter jejuni using the dose
### response function, dose response parameters and dose for that pathogen.
def pill_cj(fib_dens,animalsource):
  return drf_hyp_t20(cj_inf_dist,cj_ill_dist,dose(fib_dens,'cj',animalsource))

### The function pill_s computes the probability of illness from exposure to salmonella using the dose response function,
### dose response parameters and dose for that pathogen.
def pill_s(fib_dens,animalsource):
  return drf_bp(drp_s['a'],drp_s['b'],drp_s['c_pill'],dose(fib_dens,'s',animalsource))

### The function pill_g computes the probability of illness from exposure to giardia using the dose response function,
### dose response parameters and dose for that pathogen.
def pill_g (fib_dens,animalsource):
  return drf_exp(drp_g['r'],drp_g['c_pill'],dose(fib_dens,'g',animalsource))

### The function pill_cr computes the probability of illness from exposure to cryptosporidium using the dose response
### function, dose response parameters and dose for that pathogen.
def pill_cr (fib_dens,animalsource):
  return drf_exp(drp_cr['r'],drp_cr['c_pill'],dose(fib_dens,'cr',animalsource))

### The function pill_c57 computes the probability of illness from exposure to e-coli O157:H7 using the dose response
### function, dose response parameters and dose for that pathogen.
def pill_c57(fib_dens,animalsource):
  return drf_bp(drp_c57['a'],drp_c57['b'],drp_c57['c_pill'],dose(fib_dens,'c57',animalsource))
```

```
### The function pill_nor computes the probability of illness from exposure to norovirus using the dose response
### function, dose response parameters and dose for that pathogen.
def pill_nor(fib_dens,animalsource):
  return drf_hyp_t20(nor_inf_dist,nor_ill_dist,dose(fib_dens,'nor',animalsource))

### The function tpill computes the probability of illness from exposure to all the pathogens listed above.
### This implementation is based on equations 3J,3K, and 3L.
def tpill(fib_dens,animalsource):
  return(1-(1-pill_cj(fib_dens,animalsource))*(1-pill_s(fib_dens,animalsource))*(1-pill_g(fib_dens,animalsource))*(1-
pill_cr(fib_dens,animalsource))*(1-pill_c57(fib_dens,animalsource))*(1-pill_nor(fib_dens,animalsource)))

### The function deltarisk estimates the difference between the target risk level and the current value computed
### using an initial estimate for fib_dens
def deltarisk (fib_dens,animalsource):
  return (risk-tpill(fib_dens,animalsource))

### The following lines define the different types of animal source available in this code.
animalsource1=('swine')
animalsource2=('chicken')
animalsource3=('gulls')
animalsource4=('non-path')

### The following lines cause the initial estimate for fib_dens to be adjusted until deltarisk equals zero.
### In other words, it finds the FIB density levels that correspond to the target risk level for different
### mixtures of swine and human contamination.
fib_critical1=fsolve(deltarisk, fib_dens, args=animalsource1)
fib_critical2=fsolve(deltarisk, 0.5*fib_dens, args=animalsource2) # Note: a factor to initial estimate of fib_dens to ensure
#convergence
fib_critical3=fsolve(deltarisk, fib_dens, args=animalsource3)
fib_critical4=fsolve(deltarisk, fib_dens, args=animalsource4)
fib_critical4[fib_critical4 < 0] = nan # replace negative values in 100% non pathogenic contamination with NA

print("")
print("THE FIB RBTs in CFU/100mL CORRESPONDING TO THE TARGET RISK AT DIFFERENT LEVELS OF SWINE
CONTRIBUTION TO THE ANIMAL-HUMAN CONTAMINATION MIXTURE ARE: ") # Prints this message
print("")
print( pcfu, (fib_critical1/10).round(0))
print("")
print( "THE FIB RBTs in CFU/100mL CORRESPONDING TO THE TARGET RISK AT DIFFERENT LEVELS OF CHICKEN
CONTRIBUTION TO THE ANIMAL-HUMAN CONTAMINATION MIXTURE ARE: ") # Prints this message
print("")
print( pcfu, (fib_critical1/10).round(0))
print("")
print( "THE FIB RBTs in CFU/100mL CORRESPONDING TO THE TARGET RISK AT DIFFERENT LEVELS OF GULL
CONTRIBUTION TO THE ANIMAL-HUMAN CONTAMINATION MIXTURE ARE: ") # Prints this message
print("")
print( pcfu, (fib_critical1/10).round(0))
print("")
print( "THE FIB RBTs in CFU/100mL CORRESPONDING TO THE TARGET RISK AT DIFFERENT LEVELS OF NON-PATHOGENIC
CONTRIBUTION TO THE ANIMAL-HUMAN CONTAMINATION MIXTURE ARE: ") # Prints this message
print("")
print( pcfu, (fib_critical1/10).round(0))

### Postprocessing results. Generates output results as Excel file after appropriate transformations, transposing, and ###
### conversion to CFU/100mL to be consistent with TSM tables
import pandas as pd
```

```
hc=['10%', '20%', '25%', '30%','33%','50%', '60%','67%', '75%', '90%','100%'] # hc is an array with percent human
### contribution to fib but in reverse order compared to the nonhuman contribution array in the code above
## The lines below creates a dataframe using the arrays but reverses the order to correspond to hc, rounds them, and
### divides by 10 to generate output in CFU/100mL
df=pd.DataFrame({'% Human FIB Contribution':hc,'Human-Swine Mixture':(fib_critical1[::-1]/10).round(0),'Human-Chicken Mixture':(fib_critical2[::-1]/10).round(0),'Human-Gull Mixture':(fib_critical3[::-1]/10).round(0),'Human-Non-pathogentic Mixture':(fib_critical4[::-1]/10).round(0)})
df=df.transpose()
df.to_excel(r'C:\YourOutputFolder\RBT_Simulation_Output.xlsx',index=True,header=None)

##########QA TO ENSURE CONVERGENCE
######## IF THESE VALUES DO NOT MATCH THE TARGET RISK, ADJUST THE INITIAL ESTIMATE FOR FIB_DENS UNTIL YOU
### SEE CONVERGENCE.

print("Convergence check for ", animalsource1, tpill(fib_critical1,animalsource1))
print("Convergence check for ", animalsource2, tpill(fib_critical2,animalsource2))
print("Convergence check for ", animalsource3, tpill(fib_critical3,animalsource3))
print("Convergence check for ", animalsource4, tpill(fib_critical4,animalsource4))
```

Appendix I: Example Code for STV and BAV Calculation

This appendix provides an annotated code example for the computation of statistical threshold values (STVs) and beach action values (BAVs). This code is an example and should not be considered a tool. The computation of STVs and BAVs requires as input the magnitude of the indicator criterion in units of CFU/100 mL (the geometric mean value of the criterion) and the site-specific standard deviation (SD) of the $\log_{10}$ transformed indicator values in units of $\log_{10}$ CFU/100 mL. If the SD of the $\log_{10}$ transformed indicator values for the waterbody used in the water quality study is not available, users may use the values from the EPA's 2012 RWQC: 0.44 $\log_{10}$ CFU/100 mL for enterococci and 0.40 $\log_{10}$ CFU/100 mL for *E. coli*. This example uses enterococci as the indicator with a $\log_{10}$ SD of 0.44 $\log_{10}$ CFU/100 mL. A key for the annotated code is presented in Table I-1.

Table I-1. Key for annotated code.

KEY
BOLD—Actual R code (R available at http://www.r-project.org/)
PLAIN—Comments for guidance

```
#!/usr/bin/Rscript
#########################################################################################
#
### Example code for STV (Statistical Threshold Value) and BAV (Beach Action Value) Calculation
### Includes EPA values for log10 standard deviation of enterococci and E. coli (within annotations)
### Include examples for single use. May be modified by analysts to process a larger batch of inputs.
### This script assumes the input geometric means and log10 standard deviation of the indicator are
specified in units of CFU/100mL
#########################################################################################
#
#### Functions to compute STV and BAV
z90<-qnorm(0.90) # the z score corresponding to the 90th percentile of a normal distribution
z75<-qnorm(0.75) # the z score corresponding to the 75th percentile of a normal distribution

compute_stv<-function(GM,log10sd){ # function to compute the STV
 stv_val<- 10^(log10(GM)+log10sd*z90)
 stv_val<-round(stv_val,0)
 return(stv_val)
 }

compute_bav<-function(GM,log10sd){ # function to compute the BAV
 bav_val<-10^(log10(GM)+log10sd*z75)
 bav_val<-round(bav_val,0)
 return(bav_val)
}
```

EXAMPLES OF USE FOR A SINGLE GM

GM<-35 # user must input here the geometric mean of the indicator in units of CFU/100mL
log10sd_indicator<-0.44 ## user must input here the log10 standard deviation (standard deviation of the log10 transformed indicator data) of the indicator in units of log10 CFU/100mL or use the values from EPA RWQC 2012 of 0.44 log10 CFU/100mL for enterococci or 0.4 log10 CFU/100mL if using *E. coli* as indicator
stv<-compute_stv(GM,log10sd_indicator) # call function to compute stv
bav<-compute_bav(GM,log10sd_indicator) # call function to compute bav
print (c("The calculated STV value is ", round(stv,0)," CFU/100mL")) ## Print the calculated STV
print (c("The calculated BAV value is ", round(bav,0)," CFU/100mL")) ## Print the calculated BAV

Appendix J: Computer Code for Reverse QMRA

This appendix provides an annotated code example for replicating Soller et al. (2014) reverse QMRA. This code was used to compute the values shown in TSM Section 4.2.1. The code estimates the risk-based threshold for the enterococci level corresponding to a target illness rate for the following fecal mixtures: swine (pig)/human; chicken/human; gull/human; and nonpathogenic/human. This code can be utilized by experienced programmers after a careful reading of its syntax and structure with reference to the appropriate equations described in Soller et al. (2014) and parameters described in Soller et al. (2010b) as cited in Soller et al. (2014). This code should not be considered a tool. Note that the code outputs RBTs in units of CFU/100 mL. A key for the annotated code is presented in Table J-1 and the definitions and sources for variables for the Soller et al. (2014) reverse QMRA is presented in Table J-2.

Citations:

Soller, J.A., Schoen, M.E., Bartrand, T., Ravenscroft, J.E., Ashbolt, N.J. 2010b. Estimated human health risks from exposure to recreational waters impacted by human and non-human sources of faecal contamination. Water Research, 44(16):4674–4691.

Soller, J.A., M.E. Schoen, A. Varghese, A.M. Ichida, A.B. Boehm, S. Eftim, N.J. Ashbolt, and J.E. Ravenscroft. 2014. Human health risk implications of multiple sources of faecal indicator bacteria in a recreational waterbody. *Water Research* 66:254–264.

Teunis, P., W. Van den Brandhof, M. Nauta, J. Wagenaar, H. Van den Kerkhof, and W. Van Pelt. 2005. A reconsideration of the *Campylobacter* dose-response relation. *Epidemiology and Infection* 133(4):583–592.

Teunis, P., W. Van den Brandhof, M. Nauta, J. Wagenaar, H. Van den Kerkhof, and W. Van Pelt. 2005. A reconsideration of the *Campylobacter* dose-response relation. *Epidemiology and Infection*

Table J-1. Key for annotated code.

KEY
BOLD—Actual code for Python (software available at http://www.python.org/getit/)
PLAIN—Comments for guidance

Table J-2. Soller et al. (2014) reverse QMRA: definitions and sources for variables in equations.

In-text variable	Code variable X represents the pathogen of interest	Definition
$C_{rp}^{NHS}, C_{rp}^{HS}$	No explicit variable defined. Computation embedded within the *dose* function for program efficiency.	Estimated waterbody density of reference pathogen (number of pathogens or genomes/L) in nonhuman and human sources.
p_{FIB}^{NHS}	pcfu	Proportion of FIB density attributable to the nonhuman source *NHS* (unitless; evaluated here as a vector assuming multiple values)
C_{FIB}	fib_dens	Initial estimate of critical FIB density (CFU/L) corresponding to the target probability of illness for each proportion.
R_{rp}^{NHS}	xx_dens['*PathogenName'*]	Density of reference pathogens (number of pathogens or genomes/L or number of pathogens or genomes/g) in the nonhuman source *NHS*
R_{FIB}^{NHS}	xx_dens['ent']	Density of FIB (CFU/L or CFU/g) in the nonhuman source *NHS*
R_{rp}^{raw}	hum_raw_dens['nor']	Density of each reference pathogen (number of pathogens or genomes/g) in raw human contamination;
R_{rp}^{WWTP}	hum_wwtp_dens['nor']	Density of each reference pathogen (number of pathogens or genomes/L) in WWTP-treated human contamination;
R_{FIB}^{raw}	hum_raw_dens['ent']	Density of FIB (CFU/g) in raw human contamination
R_{FIB}^{WWTP}	hum_wwtp_dens['ent']	Density of FIB (CFU/L) in WWTP-treated human contamination
$\mu_{rp}^{NHS}, \mu_{rp}^{HS}$	dose['*fib_dens'*,'*PathogenName'*]	Estimated dose ingested of reference pathogen from nonhuman and human sources respectively (number of pathogens). Common implementation in *dose* function.
V	vol_ing	Volume of water ingested (L)
p_{rp}^{NHS}	sw_frinf['*PathogenName'*]	Fraction of human infectious pathogenic strains (unitless)
I_{rp}^{NHS}	sw_prev['*PathogenName'*]	Prevalence of infection in the nonhuman source (unitless)
$p_{ill\|inf}^{rp}$	drp_X['c_pill']	Proportion of infections resulting in illness (unitless)
f_{d-r}^{rp}	drf_bp,drf_exp,drf_hyp. Dose-response parameters for these functions are found in drp_X.	Dose-response functions for various pathogens (unitless)
$P_{ill}^{rp,NHS}, P_{ill}^{rp,HS}$	pill_X. (No explicit superscripting was required for NHS and HS because the pathogens found in the two sources did not overlap.)	Probability of illness from a specific reference pathogen rp originating from the nonhuman source NHS and human source HS, respectively (unitless)
$P_{ill}^{NHS}, P_{ill}^{HS}$	Directly implemented in *tpill* function below.	Estimated probability of illness from the nonhuman source NHS and human source HS, respectively (unitless)

In-text variable	Code variable X represents the pathogen of interest	Definition
P_{ill}	tpill	Estimated total probability of illness accounting for exposure to both the nonhuman and human sources of contamination (unitless)

```
# -*- coding: utf-8 -*-
"""
Created on Thu Apr 28 09:41:12 2022

"""
#!/usr/local/bin/python

##########################################################################################
### This is code replicating Soller et al. (2014) "reverse" QMRA. Determines FIB density
### corresponding to specified level of risk for contamination mixtures deriving
### from human and alternative animal (swine, chicken, gull, non-pathogenic) sources.

##########################################################################################

from numpy import *          ### Imports Python NumPy module
from scipy.optimize import fsolve  ### Imports necessary sub-module from Python SciPy module
from scipy.special import hyp1f1  ### Imports necessary sub-module from Python SciPy module
print ("REVERSE QMRA EXAMPLE FOR ANIMAL AND HUMAN MIXTURES") ## Prints output message
print("")
print( "FINDS FIB DENSITY CORRESPONDING TO SPECIFIED LEVEL OF RISK") ## Prints output message
print("")

risk=0.036   ### Target illness rate from the 2012 RWQC, specified as a probability.
       ### In this case, the chosen level of risk corresponds to 36 illnesses per 1000 swim events (unitless).
vol_ing=0.019  ### Volume of water ingested during the recreational event, in L. General population median value from
###### Dufour et al. 2017.

### The following lines create arrays of FIB and pathogen densities found in swine, chicken and gull contamination
### based on the midpoint of the range of values in Soller et al. (2010b) as cited in Soller et al. (2014)
### Array of FIB (cfu/g) and pathogen (organisms/g) densities found in swine contamination
sw_dens={'ent':10**((5.3+7.2)/2.0),'cj':10**((2.0+5.7)/2.0),'g':10**((0+6.8)/2.0),'s':10**((2.8+4.9)/2.0),'cr':10**((1.7+3.6)
/2.0),'c57':10**((0.0+7.0)/2.0)}
### Array of FIB (cfu/g) and pathogen densities (organisms/g) found in chicken contamination
ch_dens={'ent':10**((5.0+7.0)/2.0),'cj':10**((2.8+6.5)/2.0),'g':0,'s':10**((-1.0+4.5)/2.0),'cr':0,'c57':0}
### Array of FIB (cfu/g) and pathogen (organisms/g) densities found in gull contamination
gu_dens={'ent':10**((6.0+8.0)/2.0),'cj':10**((3.3+6.0)/2.0),'g':0,'s':10**((2.3+9.0)/2.0),'cr':0,'c57':0}

### Array of FIB (cfu/L) and pathogen (organisms/L) densities found in non-pathogenic contamination.
### Artificial density value used for enterococci density to avoid 0/0 error when calculating path::fib ratio.
np_dens={'ent':0.1,'cj':0,'g':0,'s':0,'cr':0,'c57':0}
### Nested dictionary of animal source pathogen and FIB densities.
dens_source={'swine':sw_dens,'chicken':ch_dens,'gulls': gu_dens,'non-path':np_dens}

### The following lines create arrays of pathogen prevalence rates for swine, chicken and gull contamination
### based on the midpoint of the range of values in Soller et al. (2010b) as cited in Soller et al. (2014)
### Array of pathogen prevalence rates for swine contamination (unitless)
sw_prev={'cj':(0.46+0.98)/2,'g':(0.033+0.18)/2.0,'s':(0.079+0.15)/2.0,'cr':(0+0.45)/2.0,'c57':(0.001+0.12)/2.0}
### Array of pathogen prevalence rates for chicken contamination (unitless)
ch_prev={'cj':(0.57+0.69)/2,'g':0,'s':(0.0+0.95)/2.0,'cr':(0.06+0.27)/2.0,'c57':0}
```

```
### Array of pathogen prevalence rates for gull contamination (unitless)
gu_prev={'cj':1.0,'g':0,'s':1.0,'cr':0,'c57':0}

### Array of pathogen prevalence rates for non-pathogenic contamination (unitless)
np_prev={'cj':0.0,'g':0,'s':0.0,'cr':0,'c57':0}
### Nested dictionary of pathogen prevalence rates.
prev_source={'swine':sw_prev,'chicken':ch_prev,'gulls':gu_prev,'non-path':np_prev}

### The following lines create arrays of pathogen infectious fraction estimates for swine, chicken and
### gull contamination based on Soller et al. (2010b) as cited in Soller et al. (2014)
### Array of pathogen infectious fraction estimates for swine contamination (unitless).
sw_frinf={'cj':0.833,'g':0.833,'s':0.5,'cr':0.167,'c57':0.833}
### Array of pathogen infectious fraction estimates for chicken contamination (unitless).
ch_frinf={'cj':0.5,'g':0,'s':0.5,'cr':0.167,'c57':0}
### Array of pathogen infectious fraction estimates for gull contamination (unitless).
gu_frinf={'cj':0.167,'g':0,'s':0.167,'cr':0,'c57':0}
### Array of pathogen infectious fraction estimates for non-pathogenic contamination (unitless)
np_frinf={'cj':0.0,'g':0,'s':0.0,'cr':0,'c57':0}
### Nested dictionary of infectious fraction rates.
frinf_source={'swine':sw_frinf,'chicken':ch_frinf,'gulls':gu_frinf,'non-path':np_frinf}

### The following line creates an array of FIB and pathogen densities found in raw human contamination
### based on the midpoint of the range of values in Soller et al. (2010b) as cited in Soller et al. (2014)
### Array of FIB (cfu/L) and pathogen (organisms/L) densities found in raw human contamination
hum_raw_dens={'ent':10**((5.8+8.0)/2.0),'nor':10**((3.0+6.0)/2.0)}

### Norovirus attenuation level achieved in WWTP plants based on the midpoint of the range of values in Soller et al.
(2010b) as cited in Soller et al. (2014)
wwtp_atten=10**((2.23+3)/2)

### The following line creates an array of FIB and pathogen densities found in WWTP-treated human contamination based
### on the midpoint of the range of values in Soller et al. (2010b) as cited in Soller et al. (2014) and the attenuation value
specified in the line above.
### Array of FIB (cfu/L) and pathogen (organisms/L) densities found in WWTP-treated human contamination
hum_wwtp_dens={'ent':10**((0.5+2.7)/2.0),'nor':hum_raw_dens['nor']/wwtp_atten}

### Ratio of pathogen to FIB levels in raw human contamination
ratio_hum_raw=hum_raw_dens['nor']/hum_raw_dens['ent']
### Ratio of pathogen to FIB levels in WWTP-treated human contamination
ratio_hum_wwtp=hum_wwtp_dens['nor']/hum_wwtp_dens['ent']

### Ratio of pathogen to FIB levels in mixture of raw and WWTPtreated human contamination. The fractions of raw and
WWTP
### contamination in the mixture were determined such that an ingestion volume of 0.019 L results in an illness rate
### of 36/1000 and an FIB level of 35 cfu/100mL.
ratio_dens_hmix=0.9901*ratio_hum_raw+0.0099*ratio_hum_wwtp

### Array specifies alternative levels of non-human source contribution of total FIB level in surface water. For
### instance, 0 corresponds to 100% human contribution; 0.1 corresponds to 10% non-human source and 90% human
### contribution, etc.
pcfu=array([0,0.1,0.2,0.3,0.4,0.5,0.6,0.7,0.8,0.9])

### Array contains an initial estimate of FIB levels in CFU/L corresponding to specified risk for the different
### percentages of the mixture defined in the line above.
fib_dens=array([100,100,100,100,100,100,100,100,100,100])
```

```
### The function "dose" defined below computes the dose of the specified pathogen ingested during a swim event by
### estimating the % of FIB from the source in which that pathogen is found, multiplying it by the ratio of pathogens
### per FIB in that source, and then multiplying that by the volume of water ingested.
### This implementation is based on Soller et al. (2014) Equation 1.
def dose(fib_dens,PathogenName,AnimalSource):
  if PathogenName=='nor':
    result = (1-pcfu)*fib_dens*ratio_dens_hmix*vol_ing
  else:
    result =
pcfu*fib_dens*dens_source[AnimalSource][PathogenName]/dens_source[AnimalSource]['ent']*prev_source[AnimalSou
rce][PathogenName]*frinf_source[AnimalSource][PathogenName]*vol_ing
  return result

### Beta Poisson Dose Response Function based on Table 1 in Soller et al. (2014)
def drf_bp(a,b,c_pill,d):
  return (1-(1+d/b)**-a)*c_pill

### Exponential Dose Response Function based on Table 1 in Soller et al. (2014)
def drf_exp (r,c_pill,d):
  return(1-exp(-r*d))*c_pill

### 1F1 Confluent Hypergeometric Dose Response Function based on Table 1 in Soller et al. (2014)
def drf_hyp(alpha,beta,c_pill,d):
  return(1-hyp1f1(alpha,(alpha+beta),-d))*c_pill

### Dose Response Parameters for campylobacter jejuni based on Table 1 in Soller et al. (2014) and Teunis dose dependent
### conditional probability of illness
drp_cj={'alpha':0.024,'beta':0.011,'nu':3.63*10**-9,'r':2.44*10**8}
### The following function computes the conditional probability of illness given infection for cj based on the
### Teunis dose dependent relationship
def c_pill_cj_dd(fib_dens,PathogenName,AnimalSource):
  return (1-(1+drp_cj['nu']*dose(fib_dens,PathogenName,AnimalSource))**-drp_cj['r'])

### Dose Response Parameters for salmonella based on Table 1 in Soller et al. (2014)
drp_s={'a':0.3126,'b':2884,'c_pill':(0.17+0.40)/2.0}
### Dose Response Parameters for giardia based on Table 1 in Soller et al. (2014)
drp_g={'r':0.0199,'c_pill':(0.2+0.7)/2.0}
### Dose Response Parameters for cryptosporidium based on Table 1 in Soller et al. (2014)
drp_cr={'r':0.09,'c_pill':0.5}
### Dose Response Parameters for e-coli O157:H7 based on Table 1 in Soller et al. (2014)
drp_c57={'a':0.248,'b':48.8,'c_pill':(0.2+0.6)/2.0}
### Dose Response Parameters for norovirus based on Table 1 in Soller et al. (2014)
drp_nor={'alpha':0.04,'beta':0.055,'c_pill':0.6}

### The following functions are based on Soller et al. (2010b) Table 3 and Teunis et al. (2005)
### The function pill_cj computes the probability of illness from exposure to campylobacter jejuni using the dose
### response function, dose response parameters and dose for that pathogen.
def pill_cj(fib_dens,animalsource):
  return drf_hyp(drp_cj['alpha'],drp_cj['beta'],c_pill_cj_dd(fib_dens,'cj',animalsource),dose(fib_dens,'cj',animalsource))

### The function pill_s computes the probability of illness from exposure to salmonella using the dose response function,
### dose response parameters and dose for that pathogen.
def pill_s(fib_dens,animalsource):
  return drf_bp(drp_s['a'],drp_s['b'],drp_s['c_pill'],dose(fib_dens,'s',animalsource))

### The function pill_g computes the probability of illness from exposure to giardia using the dose response function,
```

```
### dose response parameters and dose for that pathogen.
def pill_g (fib_dens,animalsource):
  return drf_exp(drp_g['r'],drp_g['c_pill'],dose(fib_dens,'g',animalsource))

### The function pill_cr computes the probability of illness from exposure to cryptosporidium using the dose response
### function, dose response parameters and dose for that pathogen.
def pill_cr (fib_dens,animalsource):
  return drf_exp(drp_cr['r'],drp_cr['c_pill'],dose(fib_dens,'cr',animalsource))

### The function pill_c57 computes the probability of illness from exposure to e-coli O157:H7 using the dose response
### function, dose response parameters and dose for that pathogen.
def pill_c57(fib_dens,animalsource):
  return drf_bp(drp_c57['a'],drp_c57['b'],drp_c57['c_pill'],dose(fib_dens,'c57',animalsource))

### The function pill_nor computes the probability of illness from exposure to norovirus using the dose response
### function, dose response parameters and dose for that pathogen.
def pill_nor(fib_dens,animalsource):
  return drf_hyp(drp_nor['alpha'],drp_nor['beta'],drp_nor['c_pill'],dose(fib_dens,'nor',animalsource))

### The function tpill computes the probability of illness from exposure to all the pathogens listed above.
### This implementation is based on Soller et al. (2014) Section 2.1.4.
def tpill(fib_dens,animalsource):
  return(1-(1-pill_cj(fib_dens,animalsource))*(1-pill_s(fib_dens,animalsource))*(1-pill_g(fib_dens,animalsource))*(1-
pill_cr(fib_dens,animalsource))*(1-pill_c57(fib_dens,animalsource))*(1-pill_nor(fib_dens,animalsource)))

### The function deltarisk estimates the difference between the target risk level and the current value computed
### using an initial estimate for fib_dens
def deltarisk (fib_dens,animalsource):
  return (risk-tpill(fib_dens,animalsource))

### The following lines define the different types of animal source available in this code.
animalsource1=('swine')
animalsource2=('chicken')
animalsource3=('gulls')
animalsource4=('non-path')

### The following lines cause the initial estimate for fib_dens to be adjusted until deltarisk equals zero.
### In other words, it finds the FIB density levels that correspond to the target risk level for different
### mixtures of swine and human contamination.
fib_critical1=fsolve(deltarisk, fib_dens, args=animalsource1)
fib_critical2=fsolve(deltarisk, fib_dens, args=animalsource2)
fib_critical3=fsolve(deltarisk, fib_dens, args=animalsource3)
fib_critical4=fsolve(deltarisk, fib_dens, args=animalsource4)
fib_critical4[fib_critical4 < 0] = nan # replace negative values in 100% non pathogenic contamination with NA

print("")
print("THE FIB RBTs in CFU/100mL CORRESPONDING TO THE TARGET RISK AT DIFFERENT LEVELS OF SWINE
CONTRIBUTION TO THE ANIMAL-HUMAN CONTAMINATION MIXTURE ARE: ") # Prints this message
print("")
print( pcfu, (fib_critical1/10).round(0))
print("")
print( "THE FIB RBTs in CFU/100mL CORRESPONDING TO THE TARGET RISK AT DIFFERENT LEVELS OF CHICKEN
CONTRIBUTION TO THE ANIMAL-HUMAN CONTAMINATION MIXTURE ARE: ") # Prints this message
print("")
print( pcfu, (fib_critical1/10).round(0))
print("")
```

```
print( "THE FIB RBTs in CFU/100mL CORRESPONDING TO THE TARGET RISK AT DIFFERENT LEVELS OF GULL
CONTRIBUTION TO THE ANIMAL-HUMAN CONTAMINATION MIXTURE ARE: ") # Prints this message
print("")
print( pcfu, (fib_critical1/10).round(0))
print("")
print( "THE FIB RBTs in CFU/100mL CORRESPONDING TO THE TARGET RISK AT DIFFERENT LEVELS OF NON-PATHOGENIC
CONTRIBUTION TO THE ANIMAL-HUMAN CONTAMINATION MIXTURE ARE: ") # Prints this message
print("")
print( pcfu, (fib_critical1/10).round(0))

### Postprocessing results. Generates output results as Excel file after appropriate transformations, transposing, and
###conversion to CFU/100mL to be consistent with TSM tables
import pandas as pd
hc=['10%', '20%', '30%','40%','50%', '60%','70%', '80%', '90%','100%'] # hc is an array with percent human contribution to
fib but in reverse order compared to the nonhuman contribution array in the code above
### The lines below creates a dataframe using the arrays but reverses the order to correspond to hc, rounds them, and
###divides by 10 to generate output in CFU/100mL
df=pd.DataFrame({'% Human FIB Contribution':hc,'Human-Swine Mixture':(fib_critical1[::-1]/10).round(0),'Human-
Chicken Mixture':(fib_critical2[::-1]/10).round(0),'Human-Gull Mixture':(fib_critical3[::-1]/10).round(0),'Human-Non-
pathogentic Mixture':(fib_critical4[::-1]/10).round(0)})
df=df.transpose()
df.to_excel(r'C:\YourOutputFolder\RBT_Simulation_Output_Soller2014_Method.xlsx',index=True,header=None)

######### QA TO ENSURE CONVERGENCE
######### IF THESE VALUES DO NOT MATCH THE TARGET RISK, ADJUST THE INITIAL ESTIMATE FOR FIB_DENS UNTIL YOU
######### SEE CONVERGENCE.

print("Convergence check for ", animalsource1, tpill(fib_critical1,animalsource1))
print("Convergence check for ", animalsource2, tpill(fib_critical2,animalsource2))
print("Convergence check for ", animalsource3, tpill(fib_critical3,animalsource3))
print("Convergence check for ", animalsource4, tpill(fib_critical4,animalsonurce4))
```