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Review of quantitative microbial risk assessments for potable water reuse†

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Potable water reuse is becoming more common as communities deal with increased water demands and climate change. Understanding the risks associated with potable reuse is essential to ensuring that public health is protected from waterborne pathogens. This paper provides a review on the studies that have performed quantitative microbial risk assessments (QMRAs) on potable reuse. The 30 articles included here studied direct potable reuse (DPR), indirect potable reuse (IPR), and/or *de facto* reuse (DFR), and a variety of pathogens, including norovirus, adenovirus, *Cryptosporidium*, *Giardia*, *Campylobacter*, and *Salmonella*. The QMRAs were either 'top-down' or regulations-focused, where log reduction targets (LRTs) were determined based on initial (*e.g.*, raw wastewater) pathogen concentrations and risk goals (*e.g.*, 10^{-4} annual risk benchmark), or 'bottom-up' or risk-estimation-focused, where risks were calculated based on known pathogen concentrations and observed/credited log reduction values (LRVs). Some studies incorporated process failures and pathogen decay, which were often a driving factor for risk, but several studies omitted one or both. Many studies compared multiple treatment trains (*e.g.*, carbon-based advanced treatment (CBAT) *vs.* reverse-osmosis-based advanced treatment (RBAT)). They found that treatment-based differences were pathogen-dependent because certain processes are better able to inactivate or remove certain pathogens. Many factors influence the risks reported in the various studies, including the assumed ratios of gene copies to infectious units (GC : IU), assumptions related to ingestion volume and frequency, dynamic *vs.* static modeling, and Bayesian approaches. The LRTs for the top-down QMRAs varied within and between studies, depending partially on the pathogen concentrations used and whether redundancy was included. The key findings from this review were that while QMRAs often have different goals warranting different assumptions, it is essential that researchers report these assumptions and their justifications so that policymakers and regulators fully understand their implications to avoid overly stringent or nonprotective regulations.

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Water impact

We conducted a comprehensive literature review on quantitative microbial risk assessments (QMRAs) for potable reuse, which will likely become more necessary due to climate change and drought. This review provides timely and critical insights into potable reuse QMRAs to inform future research and policy development for water reuse by identifying gaps, challenges, and best practices in conducting and reporting QMRAs.

1. Introduction

There has been increased interest in water reuse, particularly in the United States (U.S.), due to population growth, urbanization, climate change, and drought. Recycled water can be utilized for different ends, including non-potable reuse (*e.g.*, industrial applications, toilet flushing, irrigation[1]) or potable reuse. Indirect potable reuse (IPR) involves the planned discharge of recycled water to an environmental buffer, such as an aquifer, river, or lake, before being treated and used as drinking water.[2] For direct potable reuse (DPR), water is treated and added to the drinking water system through raw water augmentation (upstream of the drinking water treatment plant) or treated water augmentation (into the distribution system). DPR through raw water augmentation is sometimes conceptually similar to IPR, specifically when an environmental buffer with a short residence time is used. However, regulatory frameworks may

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specify the minimum amount of time that recycled water must spend in an environmental buffer to qualify as IPR. Finally, *de facto* reuse (DFR) occurs when there is unplanned or incidental wastewater influence on a community's drinking water source. DFR is relatively common, with 25% of U.S. drinking water treatment plants (DWTPs) serving more than 10 000 people having more than 1% treated wastewater in their drinking water source under normal streamflow conditions—and up to 100% at certain DWTPs during drought conditions.[3]

Prior to introducing potable reuse in communities, it is essential to assess the risks associated with waterborne diseases that could be acquired through this process. Quantitative microbial risk assessment (QMRA) is a tool commonly used to assess the likelihood of infection and/or illness resulting from pathogen exposure. The four steps include hazard identification, exposure assessment, dose–response modeling, and risk characterization.[4,5] While QMRA has been used extensively to analyze the risk of non-potable reuse including agricultural reuse or other purpose-driven applications,[6–11] there have been fewer studies on potable reuse.

As potable reuse regulatory development and project implementation occur, it is essential to understand the microbial risks presented by these systems, including how they can be estimated and ultimately managed. Therefore, the goal of this study was to review the studies that have used QMRA to assess the risks from potable reuse and highlight the implications of various assumptions made during the risk assessment. QMRAs are inherently a product of their assumptions, and if those assumptions are not clear, a QMRA can be misinterpreted. This review will also examine the pathogens driving risks, highlight risk mitigation strategies expected to be most effective, and compare log reduction targets (LRTs) and log reduction value (LRV) assumptions from different studies, as these affect the development of regulations.

2. Materials and methods

A search was performed on the Web of Science on October 28th, 2024. The search term was "ALL = (QMRA OR quantitative microbial risk assessment) AND ALL = (water reuse OR potable reuse OR recycled water OR reclaimed water)". This resulted in a total of 254 abstracts which were screened to exclude papers that focused on non-potable reuse or did not perform a QMRA. After screening, 28 of these papers were selected for comparison and analysis.

During the review of the selected papers, two additional papers were identified that did not use the term quantitative microbial risk assessment, likely because they were published before QMRA was a common term; however, these resources performed a QMRA on potable reuse.[12,13] This brought the total number to 30 studies of QMRA for potable reuse.

3. Results and discussion

3.1 Summary of studies

Nappier *et al.*[14] wrote a review summarizing epidemiological studies and QMRAs for potable reuse. The epidemiological studies found no negative health impacts associated with potable reuse,[14–16] though data were limited. Since 2018, there have been several more studies published on QMRA for potable reuse, and some have influenced the creation of LRTs for potable reuse treatment trains, such as California's recently drafted DPR regulations.[17] Therefore, the goal of this study was to provide an updated critical review on QMRA for potable reuse.

Table S1† summarizes the studies which have performed QMRA for potable reuse. It includes the target pathogens for each QMRA, the type of potable reuse project (DPR, IPR, and/or DFR), the associated treatment train(s), and the QMRA approach (*i.e.*, top-down or regulations-focused *vs.* bottom-up or risk-estimation-focused). Top-down QMRAs aim to identify LRTs based on initial (*e.g.*, raw wastewater) pathogen concentrations and assumed risk goals (*e.g.*, 10^{-4} annual risk benchmark). Bottom-up QMRAs estimate risk based on known pathogen concentrations and LRVs achieved by or credited to the treatment train, with the conservative practice of LRV crediting generally resulting in greater estimated risks. Those calculated risks are typically compared against a risk benchmark to determine whether the system is adequately protective of public health. These risk benchmarks are often based either on a probability of infection (P_{inf}), with a typical target of $<10^{-4}$ infections per person per year (pppy), or a metric that considers health outcomes (*e.g.*, disability adjusted life years (DALYs)), with a typical target of $<10^{-6}$ DALYs pppy.[18]

Table S1† also includes other factors that impact the risk calculation, including the volume of water consumed and ingestion frequency. If the pathogen concentrations used in a QMRA are based on molecular methods (*i.e.*, polymerase chain reaction (PCR)), the number of gene copies (GC) often need to be converted to infectious units (IU) for the risk assessment, as dose–response models are often developed based on infectious doses. A conservative GC : IU ratio of 1.0 assumes every gene copy equates to one infectious pathogen. However, molecular methods often overestimate infectious pathogen exposure because die-off/inactivation generally does not result in a corresponding level of genome damage. Therefore, GC : IU ratios can be significantly greater than 1.0 under real-world conditions.[19] Some QMRAs incorporate failures, sensitivity analyses, and/or pathogen decay linked to retention time in the environmental buffer. Studies differ based on the dose–response curves used for a given pathogen, although some studies directly compare multiple dose–response models to understand the implications of this assumption on resulting risk estimates. The decisions researchers made in developing their QMRAs and the implications of those decisions are discussed in more detail throughout this paper.

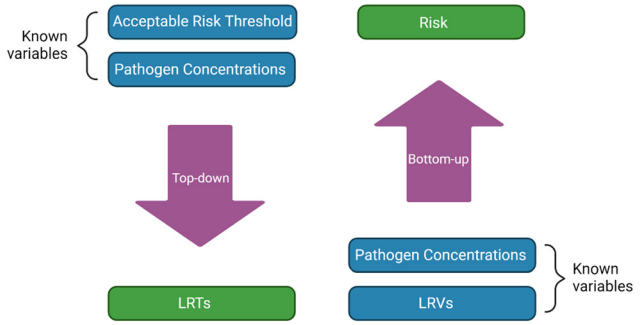


Fig. 1 Schematic of top-down *versus* bottom-up QMRAs.

3.2 QMRA type

QMRAs are typically performed top-down, where the pathogen concentrations and risk benchmarks are used to identify LRTs, or bottom-up, where the pathogens concentrations and unit treatment process LRVs are used to estimate risk (Fig. 1). The bottom-up approach was used in a number of studies to determine the risk for a certain scenario or to compare risks across multiple scenarios.[12,13,20–37] The top-down approach can be used to identify LRTs for regulatory frameworks and the unit treatment processes that might be necessary to achieve the overall LRT.[19,38–43]

Not every study performed a simple top-down or bottom-up QMRA. Two studies focused on stormwater for potable reuse and performed a blended top-down/bottom-up QMRA.[42,43] Since the studies used the same pathogen concentrations and acceptable risk threshold, they both arrived at the same LRTs. However, they then evaluated different treatment trains, specifically by varying the level of aquifer treatment, to determine if the corresponding LRVs would be sufficient to mitigate risk for the different pathogens, albeit without directly calculating risk.

MacNevin and Zornes[44] performed a bottom-up QMRA but iterated over different LRVs to determine the minimum required LRV to consistently achieve a 10^{-4} annual risk of infection, providing a similar result to following a top-down approach. They used the concentrations of *Cryptosporidium* and *Giardia* at 20 different water reclamation facilities and started with a LRV of 4. They then increased the LRV by 0.5 at each facility to determine if the annual risk of infection was less than 10^{-4} every year for 1000 simulations. This resulted in a total of 40 values of minimum LRVs, ranging from 5 to 10, for both *Cryptosporidium* and *Giardia*. MacNevin and Zornes[44] compared these results in the context of two potential treatment trains: (1) reverse-osmosis-based treatment (RBAT: UF-RO-UVAOP-ESB + Cl_2) with LRVs of 12/15/12 and (2) carbon-based advanced treatment (CBAT: O_3-BAF-UF-UVAOP-ESB + Cl_2) with LRVs of 16/16/11 for viruses, *Giardia*, and *Cryptosporidium*, respectively. All facilities would be able to surpass the LRTs with either treatment train.

Soller *et al.*[45] did much of their analysis with the bottom-up approach to determine risks for specific scenarios, but also did a top-down assessment for DPR to determine the LRTs needed to consistently meet the benchmark risk levels. They found a 14 log reduction of viruses, with norovirus as the model pathogen, and a 11+ log reduction of *Cryptosporidium* and *Giardia* resulted in around 95% of the simulations having annual risk of infection less than 10^{-4}. They demonstrated that 12/10/10 log reductions for viruses, *Giardia*, and *Cryptosporidium*, respectively, were insufficient to achieve the 10^{-4} annual risk benchmark in any of their simulations, which contradicts the findings of MacNevin and Zornes[44] for protozoa. This is potentially problematic considering that the "12/10/10" framework has been adopted for IPR in California[46,47] and Nevada,[48] and now for DPR in Colorado.[49] Differences in assumptions between MacNevin and Zornes[44] and Soller *et al.*[45] included different starting concentrations of protozoa, with MacNevin and Zornes[44] having significantly lower concentrations, the use of point-estimate LRVs associated with treatment processes[44] *vs.* uniform distributions,[45] and different dose–response models. A more recent top-down QMRA from Gerrity *et al.*[39] yielded scenarios that generally supported both Soller *et al.*[45] and MacNevin and Zornes,[44] depending on whether the pathogen concentrations were assumed to be maximum values or 97.4th percentile values, respectively.

Church *et al.*[50] used QMRA to develop tentative standards for reuse of dishwashing graywater on military bases for potable use. They followed a top-down approach, but instead of trying to determine LRTs, they determined the final maximum allowable concentrations of norovirus, *Salmonella*, and *E. coli* O157:H7 for dishwashing, showering, and drinking, without specifying a certain type of treatment. Church *et al.*[50] found that the maximum allowable concentration for potable reuse was lowest for *E. coli* O157:H7 (2.7×10^{-6} colony forming units (CFU) per mL). Since *E. coli* can be monitored with culture-based methods easily and in a cost-effective manner, Church *et al.*[50] suggested converting *E. coli* O157:H7 to total culturable *E. coli* with a ratio and applying a 10-fold safety factor. This resulted in a recommended maximum final concentration of *E. coli* of 1.6×10^{-2} CFU mL^{-1} when treating recycled dishwashing water for DPR. Overall, top-down QMRAs are useful for identifying LRTs and creating regulations, while bottom-up QMRAs can be used to evaluate the expected performance of an existing treatment train or to determine the inherent safety factor.

3.3 Potable reuse approach (DFR *vs.* IPR *vs.* DPR)

Most studies performed their assessment on only DPR[19,23,29,32,38,39,44,45,50] or *de facto* reuse/IPR.[12,13,27,28,33–37,41–43] A few studies stated they were doing IPR but neglected the impact of the environmental buffer and pathogen decay, making their analyses consistent with a DPR analysis,[26,30] although this is consistent with LRV crediting frameworks that generally omit environmental attenuation.

DPR can either utilize raw water augmentation or treated water augmentation (Fig. 2). For raw water augmentation, the treated recycled water can be added back to an environmental

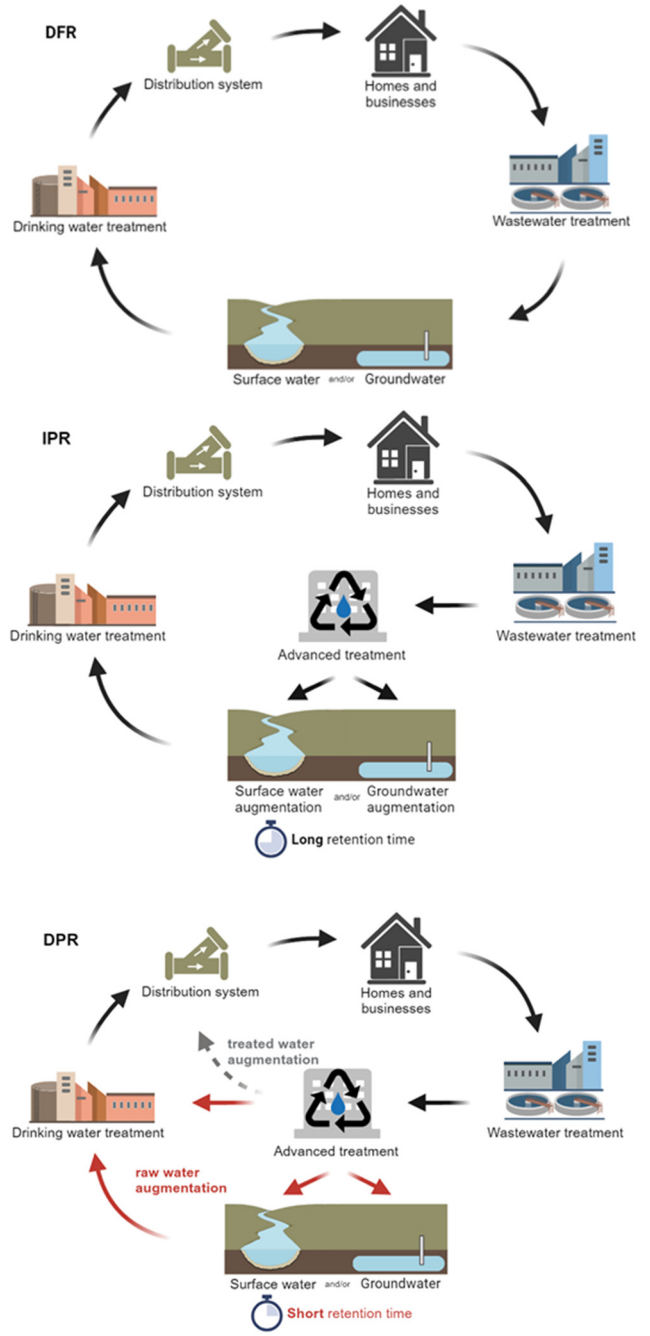


Fig. 2 Differences between DFR, IPR, and DPR (raw water augmentation and treated water augmentation).

buffer (*i.e.*, aquifer, river, or lake) upstream of a drinking water treatment plant or blended directly with the water prior to treatment. This can be distinguished from IPR based on the residence time in the environmental buffer, with some regulatory frameworks requiring minimum storage times for an IPR designation (*e.g.*, a minimum of two months in California[46]). Bailey *et al.*[23] focused their risk assessment on raw water augmentation, with a retention time of 5 days and a mixing ratio of 20% recycled water and 80% surface water. Treated water augmentation occurs when the recycled water is blended directly into the distribution system. Amoueyan *et al.*[21] studied the risks of both types of DPR. For IPR, the environmental buffer can either be surface water or groundwater, depending on the needs of a particular community. DFR is similar to IPR, but the treated wastewater at the drinking water intake is unplanned or incidental and often lacks additional/advanced treatment.

Both Soller *et al.*[31] and Amoueyan *et al.*[21] compared DFR, IPR, and DPR and found the risks of IPR and DPR to be lower than the risk of DFR if the advanced water treatment (AWT) facilities are operating within design specifications. Amoueyan *et al.*[21,22] found that the lowest risk occurred for DPR with no conventional source water (*e.g.*, surface water or groundwater), and that the risk for IPR was dominated by pathogens assumed to be present in the conventional source water (*i.e.*, not derived from local wastewater), leading to lower risks with greater recycled water contributions (RWCs). Other studies did not account for pathogen concentrations in the traditional source water and therefore found increased risk with higher percentages of recycled water.[27] Future assessments of risk in IPR systems should consider pathogen concentrations in the source water, unless there are site-specific data to support their omission, as this would allow for a fair assessment of the relative risk impact of recycled water *vs.* conventional source water. This could prevent expensive additions to the advanced treatment train on the recycled water side when the driver of risk is actually the conventional source water.

IPR is already implemented in many places, including in the U.S. in states such as California, Virginia, Texas, and Georgia, as well as outside the U.S. in South Africa, Australia, and the United Kingdom.[51] However, IPR is not a viable option for all communities, especially communities that lack access to a reservoir or aquifer with an adequate residence time or dilution ratio to sufficiently mitigate risk or meet regulatory requirements. Constructing and maintaining pipelines and pumping the treated water to reservoirs, where the water will be treated again after it is withdrawn, can be barriers for IPR implementation in some communities. Therefore, DPR may be the most sustainable option for certain communities, assuming DPR projects can be permitted. However, DPR greatly reduces the time available for detection and remediation of treatment issues (*i.e.*, the response retention time or RRT).[19] Adding an engineered storage buffer (ESB) to a DPR treatment train increases the RRT, allowing for risk reduction through mitigation of off-specification treatment.[26] This potentially increases the attractiveness of DPR from the perspective of regulators and other stakeholders.

3.4 Hazard identification: pathogens studied

The first step in QMRA involves hazard identification, which includes choosing the pathogen(s) of greatest relevance for the goals of that study. Most studies analyzed multiple pathogens, although several chose to focus on a single

pathogen.[13,20,22,25,41] This simplified the analyses and allowed for more focused evaluations, such as equivalency across reuse type (DPR, IPR, and DFR),[22] the effect of pathogen 'spikes' and hydraulic considerations on risk,[25] comparisons of static *vs.* dynamic modeling and exposure routes,[20] or how Bayesian hierarchical modeling influences parameter uncertainty with scarce data.[41] Based on the Australian Guidelines for Water Recycling, seven studies used rotavirus or adenovirus, *Cryptosporidium*, and *Campylobacter* as their pathogens.[28,35–37,40,42,43] Across the other 23 studies, norovirus and *Cryptosporidium* were the most commonly included pathogens (included in 57% and 70% of studies, respectively), and *Giardia* was also included in 43% of the studies, though Pecson *et al.*[29] did not include *Giardia* because *Cryptosporidium* was assumed to be a conservative surrogate for *Giardia*. Similarly, adenovirus sometimes required lower LRTs than norovirus and enterovirus, meaning that any LRT approach that was sufficient for controlling norovirus and enterovirus would also be sufficient for adenovirus.[19,39] Though some studies included bacteria, such as *Salmonella* and *Campylobacter*, many studies included only protozoa and/or viruses. Potable reuse regulations in the U.S. generally omit bacteria because requirements for protozoa and viruses are assumed to be highly protective against bacteria as well.[21,32] Potable reuse systems in the U.S. are also required to comply with the Safe Drinking Water Act (SDWA), which includes stipulations for bacteria.

There is still hesitance about including norovirus in QMRAs for water reuse because there are not widely used, standardized culture methods to measure norovirus infectivity, and there is uncertainty around how to utilize molecular (*e.g.*, qPCR) norovirus concentrations.[26,29] Moreover, there are multiple dose–response models for norovirus that provide different results, and there is no consensus on which is most appropriate. As can be seen in Fig. 3, the same dose of norovirus (100 infectious units) can result in an order of magnitude difference in the probability of infection. At this dose, the dose–response functions without an aggregation parameter predict the following probabilities of infection: the hypergeometric $_1F_1$ model[52] predicts 53%, the fractional Poisson[53] predicts 72%, and beta-Poisson[54] predicts 14%. Meanwhile, the fractional Poisson with an aggregation parameter[53,55,56] predicts only 6%. The goal of an aggregation parameter is to prevent overestimation of infection by accounting for incomplete mixing of norovirus with a water body, which was observed in the inoculum used in a human trial.[57] The drawback of including an aggregation parameter, however, is the unknown extent of aggregation or disaggregation of norovirus in environmental waters, leading some studies to consider the aggregated models as less conservative (*i.e.*, predict lower probabilities of illness). Chaudhry *et al.*[24] found that using the fractional Poisson aggregated dose–response model for norovirus resulted in three orders of magnitude lower median risk than using the disaggregated model. Soller

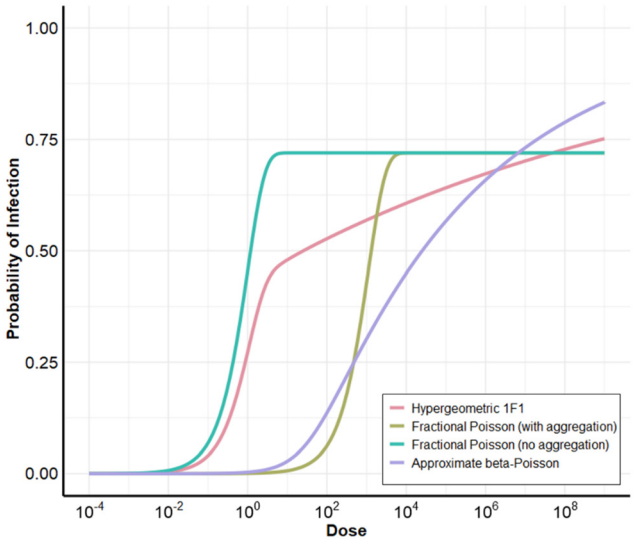


Fig. 3 Impact of norovirus dose–response model on risk: hypergeometric $_1F_1$ (no aggregation) from Teunis *et al.*,[52] fractional Poisson with aggregation from Atmar *et al.*[55,56] and Messner *et al.*,[53] fractional Poisson with no aggregation from Messner *et al.*,[53] and approximate beta-Poisson (no aggregation) from Van Abel *et al.*[54]

et al.[58] took an approach of modeling norovirus risk within two "bounds", where the lower bound was set as the aggregated fractional Poisson and the upper bound was set to the hypergeometric $_1F_1$. Lim *et al.*[27] also justified the use of the disaggregated hypergeometric $_1F_1$ as being more conservative; however, in the range simulated in Fig. 3, the fractional Poisson predicts the highest probability of infection at lower doses, indicating that the Messner *et al.*[53] model (not considered in the study) would be a potential better choice for an upper bound or conservative model. A more in-depth discussion and full comparison of norovirus dose–response models was published in Van Abel *et al.*[54] Many of the reviewed QMRAs[19,21,22,24,29,31,32,45] included multiple dose–response models for norovirus and *Cryptosporidium* due to the differences in predicted risks.

Drawbacks for norovirus inclusion in QMRAs are not limited to the dose–response model. Norovirus has multiple molecular assays that capture different strains, and some people are resistant to certain strains,[39,59] complicating the interpretation of the molecular results. Although it was included in their sampling campaign, Bailey *et al.*[23] chose not to include norovirus in their risk assessment because they did not detect any gene copies in their recycled or surface water samples. Pecson *et al.*[19] found that the uncertainty in the LRTs for norovirus spanned over 10 orders of magnitude and therefore suggested using a hybrid approach of using enterovirus occurrence data, which are culturable and in high concentrations in wastewater, and the rotavirus dose–response model, which is highly infectious, as a measure of gastrointestinal virus in reuse QMRAs.

Soller *et al.*[45] argues that norovirus should be included in risk assessments because it causes approximately 20 million illnesses a year in the U.S.,[60] more than half of the illnesses

caused by all foodborne pathogens.[61] They also argue that newer dose–response models for norovirus can capture uncertainty.[58] Soller *et al.*[45] also mentions that though norovirus is not easily culturable, the GC : IU ratios for other enteric viruses are sometimes low (*i.e.*, molecular data ≈ culture data), recently excreted viruses are likely mostly infectious, and that it is better to use conservative estimates.[62] These all support the inclusion of norovirus molecular data in reuse QMRAs, particularly when characterizing influent wastewater concentrations. In contrast, a dynamic QMRA, where community transmission was taken into account, found that waterborne norovirus likely contributes no appreciable risk to public health, because the risk for this specific organism in a community is dominated by secondary infections and foodborne transmission.[20]

Three studies have also used a surrogate enteric virus in their QMRA.[12,13,25] While Tanaka *et al.*[13] used concentrations from an enteric virus database with 377 samples from unchlorinated secondary effluents, Asano *et al.*[12] used an enteric virus database with 424 secondary effluent samples and 84 tertiary effluent samples. Gerrity *et al.*[25] used SARS-CoV-2 concentrations in wastewater with the hypergeometric dose–response model for norovirus based on the assumption that SARS-CoV-2 concentration dynamics were comparable to norovirus. While their calculated relative risks do not correspond to risk for actual pathogens, Gerrity *et al.*[25] were able to gain insights about how incidental dispersion or engineered mixing could be implemented to attenuate pathogen concentration spikes and ultimately reduce high-end risk estimates. Though Asano *et al.*[12] used the same concentrations for all the enteric viruses, they modeled the risk separately for poliovirus 1, poliovirus 3, and echovirus 12 due to different infectivities.

3.5 Pathogen concentration determination

Determining accurate pathogen concentrations for QMRAs is vital because pathogen concentration has a large impact on risk, but there are many uncertainties around what concentrations should be used. There are seasonal and geographic variations in pathogen concentrations,[63] and some studies use point estimates[12,39,40] rather than distributions. Different probability distribution functions (PDFs) could be fit to the pathogen data, such as lognormal or triangular. While most studies[24,29,31–33,45] used pathogen concentrations from raw wastewater, several used wastewater effluent data,[12,13,23,26,27,44] and a few used point estimates of pathogens in urban stormwater.[42,43] Many QMRAs assessed the sensitivity of the risk to different concentrations of pathogens in the water, often finding that they were the driving factor in risk.[12,13,21–23,33,45] Chaudhry *et al.*[24] and Soller *et al.*[45] independently used the same meta-analysis for pathogen concentrations (*i.e.*, statistical distributions of norovirus concentrations)[63] and arrived at similar risk estimates.

However, determining accurate and appropriate values can be difficult. Molecular data measures the number of gene copies, rather than the number of infectious pathogens, so the number of gene copies must be converted to infectious units (GC : IU ratios or harmonization factors). The GC : IU ratio has a large impact on risk,[19,25,64] and the numbers can vary widely. As discussed by Gerrity *et al.*,[25] conservative approaches assume a GC : IU ratio of 1.0, where every gene copy is assumed to equate to an infectious pathogen, but the actual number of infectious units might be orders of magnitude lower due to inactivation/degradation.[19] Most studies assumed all gene copies were infectious, but Bailey *et al.*[23] had percentages of infectious units for each pathogen. They used point estimates of 38.5% infectious for adenovirus (2.6 : 1 GC : IU), 65% for *Salmonella* (1.5 : 1 GC : IU), 25% for *Cryptosporidium* (4 : 1 GC : IU), and 13% for *Giardia* (7.7 : 1 GC : IU). Gerrity *et al.*[39] modeled the GC : IU ratios for norovirus, enterovirus, and adenovirus as $\log_{10}$-uniform distributions from 1 : 1 to 200 : 1, while Amoueyan *et al.*[21] used a 700 : 1 point estimate GC : IU for adenovirus. Culture methods, on the other hand, may underestimate the number of infectious viruses present. One proposed option to address this is to assume that only 10% of the viruses present are culturable,[65] and this 10-fold correction factor has recently been applied to enterovirus culture data.[19,39]

Low concentrations of pathogens can be difficult to measure, so using larger sample volumes, or more specifically larger equivalent sample volumes (ESVs),[66] can provide more data with fewer non-detects. For example, Pecson *et al.*[19] used 1 L samples to identify *Cryptosporidium* and the detection rate was 98%, compared to 40% with 50 μL samples.[67] This would not be of concern for top-down QMRAs if the LRTs are determined from the highest pathogen concentrations, but for bottom-up QMRAs using pathogen concentration distributions, the lowest concentrations would be censored and potentially omitted, resulting in overestimations of risk. The pathogen concentrations in the assessment also depend on the PDFs used to model them. Zhiteneva *et al.*[68] performed a review summarizing assumptions made when selecting the PDFs for source water, treatment steps, and the dose–response models for potable and non-potable reuse. PDFs assume variability in the system and provide a range of final risk estimates. Each dataset needs to be individually fitted to a PDF, and a poorly chosen PDF can over- or underestimate risk.

Historically, QMRAs have relied on limited data. However, the rise of wastewater surveillance for SARS-CoV-2 during the COVID-19 pandemic has caused a substantial increase in wastewater biobanks, with additional reuse-relevant pathogen datasets based on these samples being published. This increase in pathogen data highlights the importance of reviews such as Zhiteneva *et al.*[68] and Darby *et al.*[69] These papers focus on identifying and aggregating high-quality pathogen data, and they provide criteria on fitting data to distributions, guiding future pathogen data collection and selection for QMRAs.

Dispersion/mixing of pathogens in sewer collection systems and wastewater treatment plants (*e.g.*, in clarifiers and aeration basins) results in overall 'averaging' of pathogen concentrations

over time, effectively attenuating high end concentrations but also elevating low-end concentrations. This may inflate measures of central tendency by increasing risk for most ingestion events, but it will also reduce risks at the upper percentiles that often drive LRT determinations.[25] The attenuation effect is particularly apparent for intermittent spikes in influent pathogen concentration (*i.e.*, outlier events).[25] Some QMRAs use point estimates based on maximum influent pathogen concentrations, but those data points may be spikes (*i.e.*, outliers) that might actually be attenuated after accounting for dispersion. However, this benefit of dispersion is only realized with intermittent spikes; if high concentrations last for an extended period of time (*e.g.*, during a community outbreak), the effects of dispersion might be negligible. Just as there are stipulations for chemical peak averaging,[17] implementing similar guidelines for pathogens could be beneficial, considering the significant impact it could have on pathogen concentrations and resulting LRTs or credited LRVs.

3.6 Environmental buffers: retention time, pathogen decay, and recycled water contribution

Pathogens decay over time, making the inclusion of retention time in the environmental buffer an important consideration. However, many QMRAs and regulatory frameworks do not explicitly consider LRVs during storage or may only account for decay for certain pathogens. In California, for example, 1 log virus reduction is credited for each month that the water is retained underground for groundwater replenishment.[70] If pathogen decay is not considered, any corresponding risk estimates might be artificially inflated, particularly for IPR and DFR scenarios. Similarly, omitting decay from LRV crediting inevitably increases capital and operations and maintenance costs associated with engineered treatment trains, while potentially yielding no appreciable change in public health protection.[39]

The differences in decay rates for the different pathogens impact the needed retention times for risk reduction. For DFR, Amoueyan *et al.*[22] found that risk associated with wastewater-derived *Cryptosporidium* exhibited a meaningful increase with fewer than 105 days of storage in the environmental buffer, while Amoueyan *et al.*[20] found that a reservoir storage time of at least 30 days could potentially reduce risk from norovirus in a DFR system below that of DPR, using bacteriophage MS2 decay rates as a surrogate for norovirus.

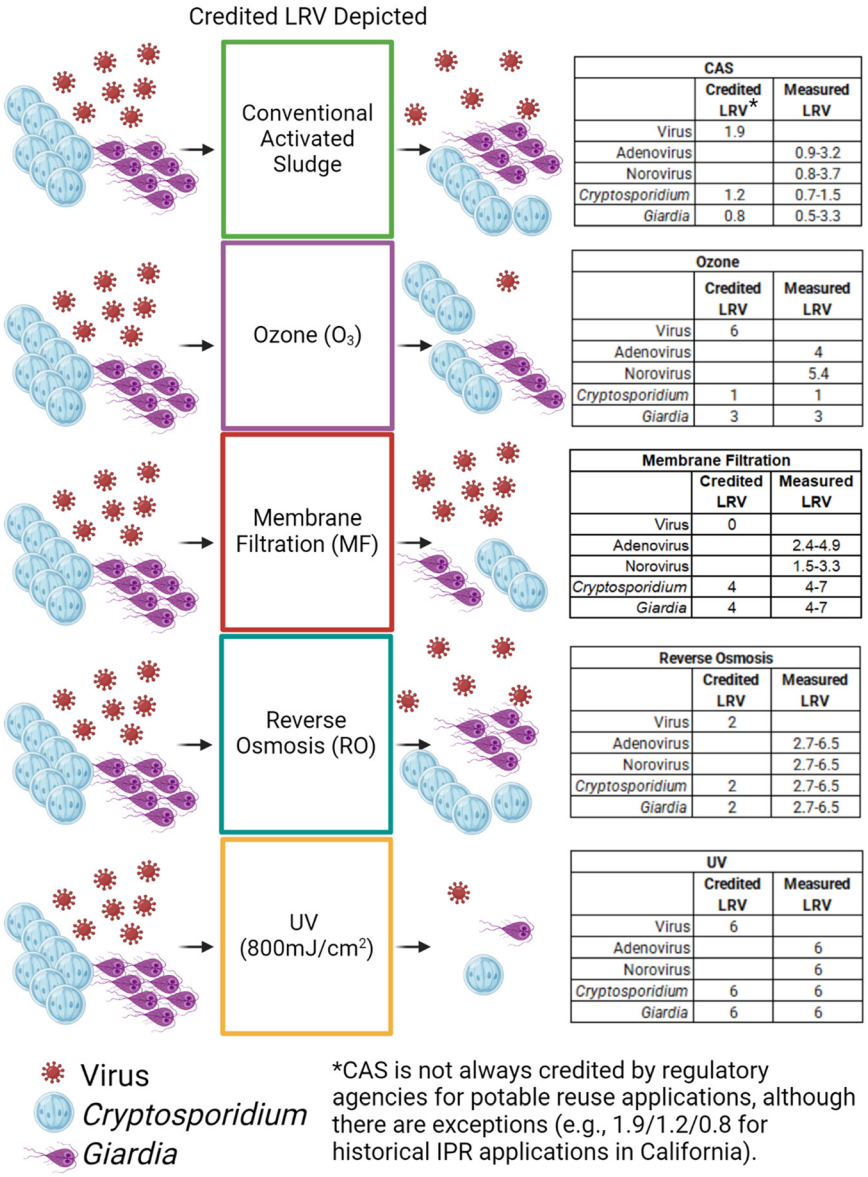
Even 1% of wastewater effluent in the drinking water source water can have important health risk considerations in reuse.[24,31] Soller *et al.*[31] included *Cryptosporidium*, *Giardia*, and norovirus in their analysis, and used residence times of 2–360 days for DFR and 30–360 days for IPR. They found that simulations with a retention time less than 180 days exceeded the annual risk benchmark of 10^{-4}, even with an RWC of 1% for DFR. With more than 10% wastewater contribution with DFR, more than 180 days were needed to consistently achieve a probability of annual infection of less than 10^{-4}. Approximately 90 days in the reservoir were required to consistently meet the annual risk benchmark of 10^{-4} for IPR with surface water augmentation. For DFR, Lim *et al.*[27] also found a negative correlation of risk with the residence time in the lake (between 270 and 360 days), and a positive correlation of risk with RWC, because they assumed the source water was pathogen free. Tanaka *et al.*[13] and Asano *et al.*[12] both assumed a residence time of 6 months in the reservoir, while Zhiteneva *et al.*[33] modeled their residence time between 50 and 120 days. In California, to be considered IPR, instead of DPR with raw water augmentation, the retention time must either be at least 180 days or the project could apply to the State Board for approval for a reduced theoretical retention time, though it can be no less than 60 days.[46]

Page *et al.*[28] studied the impact of aquifer treatment for urban stormwater in a managed aquifer recharge system. They found that the aquifer alone resulted in LRVs of 1.4, 2.6, and >6.0 for rotavirus, *Cryptosporidium*, and *Campylobacter*, respectively, based on diffusion chamber studies. They used different decay rates for the pathogens in the wetlands and the aquifer, with higher average decay rates for rotavirus and *Cryptosporidium* in the wetland, and a higher decay rate for *Campylobacter* in the aquifer. The importance of the aquifer as a treatment barrier depended on the pre- and post-treatment processes, but the estimated risk was less than 10^{-6} DALYs pppy with adequate treatment and retention time.[28]

Pathogen decay is not always included in QMRAs, even when studying DFR or IPR,[24,30] but it can have a large impact on the risk and can vary seasonally. Though Lim *et al.*[27] did not include a temperature component to their decay equations, they highlighted it as a parameter to be incorporated in future models. Bailey *et al.*[23] modeled pathogen decay at different temperatures (4 and 20 °C), but their retention time was only 5 days. Amoueyan *et al.*[21] did include the temperature component, using higher decay coefficients at higher temperatures, potentially allowing for a more accurate assessment of decay. Pathogen decay rates depend on a variety of factors including temperature, sunlight, and salinity, and the experimental decay rates for the same viral types can vary by over an order of magnitude.[71–73] However, the exact impact of these factors on decay rates of different pathogens and how they impact each other is still unknown, thus more data are needed. Collecting these data is essential because it can elucidate what LRVs could be credited for different pathogens at various retention times to help reduce reliance on engineered treatment processes by leveraging natural management barriers.

3.7 Treatment processes

Treatment processes have different LRVs for different pathogens (Fig. 4), and the pathogen that drives risk in the final estimates depends on the treatment train in question.[24,32] Soller *et al.*[32] and Chaudhry *et al.*[24] found norovirus or *Cryptosporidium* as the driving factor of risk of infection depending on the treatment processes considered.



CAS	Credited LRV*	Measured LRV
Virus	1.9	
Adenovirus		0.9-3.2
Norovirus		0.8-3.7
Cryptosporidium	1.2	0.7-1.5
Giardia	0.8	0.5-3.3

Ozone	Credited LRV	Measured LRV
Virus	6	
Adenovirus		4
Norovirus		5.4
Cryptosporidium	1	1
Giardia	3	3

Membrane Filtration	Credited LRV	Measured LRV
Virus	0	
Adenovirus		2.4-4.9
Norovirus		1.5-3.3
Cryptosporidium	4	4-7
Giardia	4	4-7

Reverse Osmosis	Credited LRV	Measured LRV
Virus	2	
Adenovirus		2.7-6.5
Norovirus		2.7-6.5
Cryptosporidium	2	2.7-6.5
Giardia	2	2.7-6.5

UV	Credited LRV	Measured LRV
Virus	6	
Adenovirus		6
Norovirus		6
Cryptosporidium	6	6
Giardia	6	6

Fig. 4 Conceptual diagram of the credited effectiveness of different treatment processes on protozoa and viruses. LRVs from Soller *et al.*[45] Note that observed treatment efficacy may be substantially different from credited treatment efficacy, resulting in an LRV 'gap'.

For example, RBAT (WWTP-MF-RO-UV-ESB + Cl_2) led to norovirus having the highest risk, while CBAT (WWTP-O_3-BAF-UF-UV-ESB + Cl_2) resulted in the risk being dominated by *Cryptosporidium*, in part because of differing observed or credited LRVs for various treatment process/pathogen combinations (Fig. 4).[32,45] In their study, Soller *et al.*[32] used data describing conventional filtration of wastewater to derive LRVs for biologically active filtration (BAF), although LRVs are not currently credited for BAF under some regulatory frameworks (*e.g.*, in California).[45] Kimbell *et al.*[34] compared two RBAT trains (TT1: BNR-MBR-RO-UV AOP-Cl_2-O_3 and TT2: BNR-MF/UF-CF-RO-UV AOP-Cl_2-O_3) and found that TT1 (14.5/14/12) achieved higher virus LRVs than TT2 (14/15.5/13.5) but lower removal of *Giardia* and *Cryptosporidium*. Amoueyan *et al.*[21] also found that whether the annual disease burden was higher for *Cryptosporidium* or norovirus depended on the treatment train.

As noted earlier, observed LRVs, which are measured experimentally and represent the actual inactivation or removal of microorganisms from the water, are often not the same as the credited or regulatory LRVs. For example, Amoueyan *et al.*[21] incorporated mean observed LRVs for microfiltration (MF) of 4.60, 2.40, and 3.65 for *Cryptosporidium*, norovirus, and adenovirus, respectively, but noted that the corresponding credited LRVs would likely be 4, 0, and 0 in an actual system. Amoueyan *et al.*[20] estimated risk for norovirus using both LRV approaches and found the risk was orders of magnitude higher using regulatory LRVs—sometimes yielding 95th percentiles exceeding 10^{-4} pppy. When lower credited LRVs result in overestimated risk to

consumers, the outcome may be overdesigned and potentially cost-prohibitive projects.

Chaudhry *et al.*[24] conducted a literature review to incorporate observed LRVs and found membrane processes were the most effective at reducing overall risk, despite UV generally being the most robust from a crediting perspective. Many potable reuse systems will employ UV doses well in excess of 200–300 mJ cm^{-2} in order to target photolysis of *N*-nitrosodimethylamine (NDMA) and/or oxidation of recalcitrant compounds such as 1,4-dioxane (*i.e.*, UV AOP), yielding LRV credits of up to 6 for all pathogen groups. In contrast, the mean LRV for UV in Chaudhry *et al.*[24] was 2.2 for *Cryptosporidium* and 5.0 for norovirus. The *Cryptosporidium* LRV[74] was based on a UV dose of 1.8 mJ cm^{-2}, and the norovirus LRV[75] was based on a UV dose of 127 mJ cm^{-2} (with MS2 as a surrogate). Since the LRV credits were limited by the lower assumed UV doses, Chaudhry *et al.*[24] found that RO—and not UV—resulted in the largest risk reduction when it was employed. When RO was not used, MF and NF reduced risk most in their treatment train. Other studies also describe the significance of UV design dose on the resulting pathogen risk. For example, Soller *et al.*[32,45] found that reducing the UV dose from 800 mJ cm^{-2} (*i.e.*, UV AOP) to 12 mJ cm^{-2} (*i.e.*, closer to traditional wastewater treatment) increased the risk of infection by four orders of magnitude, making the low dose UV treatment trains unable to meet the benchmark risk levels.

Annual risks of infection were sometimes lower for CBAT (O_3-BAF-UF-ESB + Cl_2) *vs.* RBAT (MF-RO-UV-ESB + Cl_2),[45] which was consistent with Amoueyan *et al.*[20,21] who also compared CBAT (UF-O_3-BAC-UV-ESB + Cl_2) *vs.* RBAT (MF-RO-UV-ESB + Cl_2). Although risk may have been lower with CBAT because risk incorporates both pathogen load and treatment, RBAT was sometimes superior from a treatment perspective (*i.e.*, higher overall LRVs), particularly for *Cryptosporidium*.[32,45] Ozone is a robust barrier in terms of bulk organic matter transformation, trace organic compound oxidation, and microbial inactivation,[76] yet protozoan pathogens (namely *Cryptosporidium*) still demonstrate resistance.[77] On the other hand, membrane-based treatment can be a challenge in terms of regulatory virus LRV crediting but is generally accepted as a robust barrier for protozoan pathogens from both a regulatory and observed LRV perspective (Fig. 4). Remy *et al.*[30] evaluated a unique treatment train consisting of filtration, reverse electrodialysis, micro-grain activated carbon (μGAC), and UV as advanced tertiary treatment before reservoir augmentation. They found that train consistently yielded higher risks than a more conventional potable reuse train with UF and RO with a 5% bypass. However, both treatment trains were able to meet the 10^{-6} DALY benchmark for viruses, bacteria, *Giardia*, and *Cryptosporidium*.

3.8 Exposure assessment: ingestion volume and frequency

When doing QMRA for drinking water, both the daily ingestion volume and frequency can impact the final risks.[26,29] With respect to volume, most studies assumed 2 or 2.5 L per day, but Kobayashi *et al.*[35] assumed 0.75 L d^{-1}, while Church *et al.*[50] assumed 3 L d^{-1}. More frequent ingestion events are more likely to capture rare/short-term but high consequence scenarios. However, calculated risk is somewhat attenuated when the overall daily ingestion volume is spread out over multiple ingestion events (Fig. 5). When California created their draft DPR regulations, they used a daily risk benchmark and 96 ingestion events per day. The modeled failure (assumed to be 15 minutes) would only impact one off-specification ingestion event and would be averaged out by 95 other nominal ingestion events during that day. Similarly with 96 ingestion events, a peak pathogen concentration can also be averaged out. This effectively creates a 2 log buffer (~1% of the ingestion events are impacted by failure or a pathogen spike), which would not be the case for QMRAs assuming one ingestion event per day.[39] This is also the basis for California requiring only 4 log treatment redundancy to account for a 6 log treatment process failure lasting 15 minutes.[39]

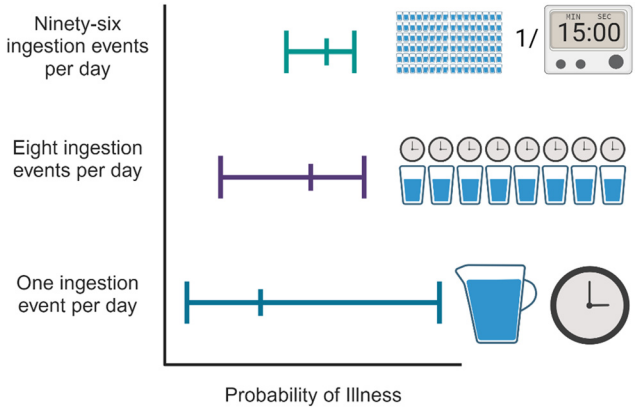


Fig. 5 Impact of number of consumption events on risk.

This phenomenon is similar to the dispersion effect discussed in Gerrity *et al.*[25] To further explore the risks with different consumption patterns, Jones *et al.*[26] modeled 1, 8, or 96 ingestion events per day, which captured different pathogen concentrations and different log reductions for each treatment process, based on different possible failure analyses. Only consuming water once per day results in a risk profile that has a larger range than when water is consumed multiple times per day. However, Jones *et al.*[26] also found higher median risks for multiple consumptions a day, again because 'averaging' has a disproportionate effect on the lower percentiles of risk.

3.9 Risk characterization

There are different ways to measure risk. Risk endpoints include probabilities of infection and illness, which can be translated into DALYs. Commonly used risk thresholds for probability of *infection* (P_{inf}) from drinking water include 10^{-4} infections per person per year (pppy) or a daily risk of 2.7×10^{-7} (*i.e.*, 10^{-4} annual risk divided equally across 365 days). The daily risk threshold is potentially more conservative, and potentially more protective of highly susceptible populations, because it

does not allow higher risk days to be averaged out, as is the case for the historically common annual risk calculation. Risk of *illness* requires an additional adjustment to account for the proportion of infections that result in a symptomatic illness. DALYs, on the other hand, are a better representation of the overall health burden of pathogens, as they measure the life years lost or lived with a disability due to pathogen exposure and subsequent infection and illness. The typical guideline is $<10^{-6}$ DALYs pppy.[18] Some studies, instead of directly using a dose–response function, used the dose equivalent to 10^{-6} DALYs for each pathogen.[40,42,43]

Using one risk endpoint *versus* another can sometimes lead to opposing conclusions. For example, Lim *et al.*[27] performed a risk assessment for norovirus and *Cryptosporidium* for DFR and found a higher risk of infection for norovirus (4.4×10^{-2} to 6.4×10^{-1} pppy) than *Cryptosporidium* (1.2×10^{-4} to 8.8×10^{-3} pppy), but a greater disease burden for *Cryptosporidium* (7.1×10^{-8} to 5.3×10^{-6} DALYs pppy) than norovirus (6.2×10^{-11} to 3.0×10^{-8} DALYs pppy). This difference is caused by the assumption that *Cryptosporidium* will have a greater negative health impact (*i.e.*, more severe) than a norovirus infection. When deciding on the preferred risk endpoint, the intended audience is an important consideration. DALYs are potentially more appropriate for communicating and comparing risks outside the U.S., as they are recommended by WHO and used globally (*e.g.*, in Australia).[35] However, regulatory development for potable reuse in the U.S. has primarily focused on probability of infection.[39]

Remy *et al.*[30] and Zhiteneva *et al.*[33] focused on the DALY framework and found risk was driven by *Cryptosporidium*. Although Remy *et al.*[30] found that *Cryptosporidium* led to higher DALY estimates than rotavirus, Page *et al.*[28] estimated higher DALYs for rotavirus than *Campylobacter* and *Cryptosporidium*. This highlights how disease burden may need to be reevaluated over time, at least in certain regions. The rotavirus vaccine RV5 was introduced in the U.S. in 2006, and the RV1 vaccine was introduced in 2008. Both vaccines are effective in reducing risk and disease burden.[78] As new vaccines are developed and dose–response models are created, the pathogens targeted by regulations may need to change to be properly representative. For example, Bailey *et al.*[23] published a QMRA in 2020 and found that adenovirus actually yielded the highest risk when compared to *Salmonella*, *Cryptosporidium*, and *Giardia*. This was due to adenovirus' higher concentrations in recycled water and surface water, presumably due to inadequate disinfection during wastewater treatment that incorporated chloramination and UV. Kimbell *et al.*[34] found that adenovirus also had the highest risk when failures were modeled, compared to a generic enteric virus, *Cryptosporidium*, and *Giardia*, though without failures, the generic enteric virus had higher average risks. In either case, viruses dominated the risk calculation because of their higher concentrations.

One study developed a unique alternative to the common risk benchmarks. Church *et al.*[50] chose a target of one illness per 50 000 exposures (daily probability of illness of 2×10^{-5} per person), meaning that if a city had 50 000 people drinking once per day, one person per day would get ill on average. This benchmark was chosen because it was two orders of magnitude less than the number of food and water-related illnesses in a military field setting, allowing reuse to contribute up to 1% of the health burden. For reference, this would be much higher (less conservative) than the aforementioned 2.7×10^{-7} daily probability of infection.

3.10 Impact of failures on risk

A single day with a peak pathogen concentration, either due to a pathogen spike or treatment process failure(s), can drive annual risk, so it is important to have reliable online monitoring.[32] Failure can be the primary driver of high risk, so it is important to stress test the failure assumptions incorporated into a QMRA.[26,33] Amoueyan *et al.*[20–22] included a probability of failure for each treatment process in the train. Amoueyan *et al.*[21] found that while DPR with treated water augmentation typically satisfied public health benchmarks, compound treatment process failures, where multiple processes fail interdependently/simultaneously, resulted in risks as high as 10^{-2}. They also performed a sensitivity analysis on treatment process failures to determine how large of an impact a failure would have on the risk from each pathogen. However, Amoueyan *et al.*[21] noted they may have overestimated the frequency and/or severity of failures (*i.e.*, LRV = 0). They also highlighted that potable reuse systems would likely have failsafe protocols, such as continuous monitoring with diversions when failures occur, and this was not incorporated into their QMRA. Similar to Amoueyan *et al.*,[21] Zhiteneva *et al.*[33] explored the failure of each treatment step by setting each LRV to 0, and they also created a model where the performance of one LRV was correlated with another by 0.5, which could be used to explore process dependency. Kimbell *et al.*[34] included three possible failure scenarios: a 3 log reduction in treatment for 24 h on 9% of simulated days, a 6 log reduction in treatment for 24 h on 1% of simulated days, and a compound 9 log reduction in treatment for 24 h occurring on 0.09% of simulated days. Without failure, one of their treatment trains had a mean P_{inf} for adenovirus of 6.65×10^{-9} pppy, but the P_{inf} reached a maximum of 7.34×10^{-2} with failures. Compound failures resulted in larger 95th or 99th percentile DALY values, depending on their frequency, demonstrating the importance of considering any correlations in process failures.

Jones *et al.*[26] compared no failure, real failure values from the literature, and total failure. Total failure of UV-AOP (which was simulated to last 15 minutes due to online monitoring and subsequent diversion to an ESB) was the largest driver for increased risk, due to its 6 log credit during normal operation. Jones *et al.*[26] found that the hypothetical failure increased the risk for higher percentile annual infection probabilities by up to six orders of magnitude, but the ESB ensured the annual risk of infection still complied with the WHO annual risk limit. Pecson *et al.*[29] assumed a maximum of one critical failure per year per process, where

the LRV for that process became 0, which was likely a conservative estimate. They reported median, 95th, and 99th percentile annual risks of infection with and without failures for *Cryptosporidium* and enterovirus. The median risks of infection without failures were 4.9×10^{-11} and 1.5×10^{-14} for *Cryptosporidium* and enterovirus, respectively. With failures, the median risks of infection increased to 1.4×10^{-7} for both *Cryptosporidium* and enterovirus, and the 99th percentiles increased to 1.1×10^{-5} and 2.1×10^{-5} for *Cryptosporidium* and enterovirus, respectively. Since Jones *et al.*[26] and Pecson *et al.*[29] were not modeling the impact of compound failures, the risks during failure events were lower than those found by Amoueyan *et al.*[21] This highlights the importance of preventing failures and ensuring any treatment train is robust and reliable.

Bailey *et al.*[23] measured pathogen concentrations in recycled water after conventional wastewater treatment and assumed a worst-case scenario for the LRV at the drinking water treatment plant using real-world data from Hijnen and Medema.[79] They compared risks from these worst-case scenarios to baseline scenarios, specifically U.S. EPA's LRVs (4/3/2 for virus/*Giardia*/*Crypto*) and the WHO's DALY-based LRVs (4/3/3 for virus/*Giardia*/*Crypto*) for conventional drinking water treatment. They found that the mean and 95th percentile annual risk for *Salmonella*, *Cryptosporidium*, and *Giardia* for the worst-case scenarios were always within an order of magnitude of the baseline conditions. For adenovirus, the mean and 95th percentile annual risk of infection was between 1 and 2 logs higher for the worst-case scenarios. Because Bailey *et al.*[23] used observed data, these worst-case scenarios had less of an impact than some of the modeled failures elsewhere in the literature (*e.g.*, Pecson *et al.*[29]).

Pecson *et al.*[19] suggested incorporating 4 log treatment redundancy to protect against undetected failures, for final LRTs of 17/14/14 for viruses/*Giardia*/*Cryptosporidium*. Gerrity *et al.*[39] assessed the NWRI Expert Panel recommendations for DPR, which included a recommended 5 log redundancy.[80] Following the Expert Panel's approach, the top-down QMRA suggested that a 5 log redundancy was sufficient to achieve a 2.7×10^{-7} daily risk benchmark at the 99th percentile, except for *Giardia* with a slightly higher daily risk.[39] Rather than incorporating redundancy, Gerrity *et al.*[39] proposed an alternative approach that quantifies a system's LRV tolerance to off-specification conditions. They found that for baseline LRVs of 15/11/11 in a DPR system, off-specification operation with an LRV of 12 for viruses or 8 for *Giardia* and *Cryptosporidium* would still satisfy the annual risk benchmark assuming the reduced LRV occurred fewer than 12 days per year for viruses or 3 days per year for the protozoa. This suggests a built-in redundancy of 3 logs for short-term off-specification conditions or failures.

Despite the potentially significant impact of failures, potable reuse treatment trains have been found to be robust and reliable. Pecson *et al.*[81] assessed the mechanical reliability of a DPR treatment train using operator logs of all mechanical issues over a year and found no critical failures, demonstrating the potential reliability of advanced treatment for DPR. Amoueyan *et al.*[22] found that some failures can be inconsequential because of the overall robustness and redundancy of advanced treatment in DPR or the resiliency afforded by the environmental buffer in IPR.

3.11 Computational methods

Most QMRAs are performed with Monte Carlo simulations to account for uncertainty and variability in the input parameters. In Monte Carlo simulations, some or all of the input variables, such as pathogen concentrations, are represented by PDFs. These statistical distributions are randomly sampled for each variable, and the outcome, such as the probability of infection, is calculated. This is repeated many times, creating a distribution of outcomes, to analyze the behavior of the system while accounting for inherent variability and uncertainty.

Simpler QMRAs can also be performed, such as by using conservative point estimates instead of distributions for pathogen concentrations. For example, Page *et al.*[40] used the 95th percentile pathogen concentrations to determine the LRTs for *Cryptosporidium*, *Campylobacter*, and viruses for urban stormwater reuse, and Gerrity *et al.*[39] used both the maximum point value and the 97.4th percentile point value from pathogen distributions. Percentile is linked to sample size, so the 97.4th percentile was chosen since this percentile within a 10 000 point dataset is statistically equivalent to the maximum value of a 24 point dataset, as can be shown from Blom's equation.[82] This might be the required minimum sample size for pathogen monitoring campaigns aimed at developing LRTs. In other words, the maximum value from a 10 000 point distribution might be considered overly conservative when compared against the maximum from a dataset with only 24 values. Asano *et al.*[12] used four point estimates for pathogen concentrations: the maximum and 90th percentile concentrations of the secondary effluent at the WWTP (assuming an additional LRV of 5 for tertiary treatment), the maximum value detected in the tertiary effluent, and the limit of detection for a tertiary treated wastewater effluent sample. Point estimates with conservative values are useful for creating point estimate regulatory LRTs, while using distributions of concentrations allow the risk distributions and central tendencies to be quantified and more fully characterized.[19]

QMRAs can also be performed dynamically or statically. In static QMRAs, the probability of infection is modeled from a single exposure event without time dependence or system feedback through community spread (Fig. 6). For waterborne diseases, static QMRAs could underestimate overall risk by not including time-dependent secondary transmission, or overestimate the risk by not including the possibility of someone entering an immune state after exposure to the waterborne pathogens.[20] While most QMRAs are static, dynamic QMRAs offer time-dependent pathogen loads and the ability to explore the relative contribution of waterborne

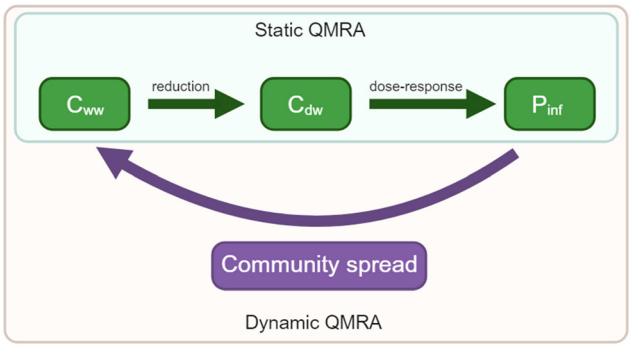


Fig. 6 Differences between dynamic and static QMRAs. C_{ww} is the pathogen concentration in wastewater. C_{dw} is the pathogen concentration in drinking water. P_{inf} is the probability of infection.

pathogens to the total number of illnesses. Amoueyan *et al.*[20] used a dynamic QMRA to determine the relative importance of norovirus transmission pathways: foodborne, person-to-person, and person-to-sewage-to-person. They modeled different epidemiological states, such as susceptible, exposed, diseased, carrier, and post-infection (or recovered) using ordinary differential equations, similar to Eisenberg *et al.*[83] Eisenberg *et al.*[83] created a dynamic process model for a *Cryptosporidium* outbreak that included a 10-state compartmental model of the population, where people could move between susceptible, infected, diseased, or immune states. The number of current infections influenced the infection rate both through person-to-person transmission and through person-to-sewage-to-person transmission. Overall, Amoueyan *et al.*[20] found that waterborne norovirus did not appreciably contribute to the public health risk in their model, because secondary and foodborne transmission dominated the overall risk calculation. Barker *et al.*[38] also included a secondary attack rate, quantifying the percentage of people who would become sick after contact with the infected person. They found that small communities might need additional treatment due to this secondary transmission and the increased contact between members in a small community relative to a large city.

Zhiteneva *et al.*[33] proposed using Bayesian networks as a solution to limited local data availability, where local pathogen data could be combined with pathogen datasets from literature reviews. Bayesian modeling uses Bayes' theorem to update the probabilities of an outcome as more information becomes available.[84] Bayesian networks are graphical models that represent a large amount of data using nodes to represent random variables that are connected to each other by their probabilistic dependencies. While Monte Carlo simulations are better suited for prediction because of their continuous distributions, Bayesian networks can be used for forward and backward inference, which could be used to determine how processes perform under certain risk scenarios.[33] Bayesian hierarchical modeling (BHM), which is better able to account for variability within and between groups of data, reduces local parameter uncertainty compared to separate modeling, where larger datasets are not taken into account, while still letting local data dominate.[41] Seis *et al.*[41] used both local and external pathogen concentrations and compared BHM to separate modeling, where each treatment plant is different and results from one do not influence results from another; complete pooling, where every treatment plant has the same mean and standard deviation; and no pooling, where the treatment plants have different means but a common standard deviation. They included a classical Bayesian hierarchical framework, where a unique mean is estimated for every treatment plant, with the assumption that the local means comes from a common, normal distribution. Seis *et al.*[41] also used extended hierarchical modeling, by letting the individual within-treatment plant variances differ by plant, which added additional hyperparameters in the model. In both cases, the parameters are all estimated on a total data and individual treatment plant level simultaneously, and the information is shared across simulations.[41] They found BHM reduced parameter uncertainty, particularly when local data were sparse, while letting local data dominate. Seis *et al.*[41] recommended including external information, such as from meta-analyses of pathogen concentrations, even when local data are available. Widespread use of Bayesian modeling for QMRA could provide more robust analyses, particularly in data-scarce scenarios, by allowing local pathogen concentrations to be supplemented by larger datasets. Bayesian modeling also enables the creation of prediction intervals, quantifying the uncertainty around the predictions. While these could be useful for a greater understanding of risks, the communication of these prediction intervals would be important to prevent unnecessary alarm or unwarranted complacency.

3.12 Regulatory considerations

Top-down QMRAs are the basis for regulations that determine the minimum LRVs for the selected pathogens. In the U.S., viruses, *Giardia*, and *Cryptosporidium* are regulated, with these values seen as inclusive for adequate bacterial removal. Table 1 shows the recommended LRTs for the top-down QMRAs on potable reuse. The LRTs varied greatly, both between and within studies. In Barker *et al.*,[38] for example, they compared an outbreak scenario for a small community to a municipal sewage scenario and found the LRT for viruses was 5.2 logs higher for an outbreak condition. The three studies with the lowest LRTs all used estimates of stormwater pathogen data.[40,42,43] MacNevin and Zornes[44] used protozoa concentrations at 20 different WWTPs and found that the LRT for *Cryptosporidium* differed by as much as 10 depending on the treatment plant in question. Using maximum pathogen concentrations, Gerrity *et al.*[39] found similar LRVs (15/11/11) as Soller *et al.*[45] did when 100% of their simulations had an annual risk less than 10^{-4}, though Soller *et al.*[45] required either 1 more virus LRV (16/11/11) or 2 more LRVs for *Cryptosporidium* and *Giardia* (15/13/13). These differences could be due in part to rounding, where Soller *et al.*[45] assessed the percentage of simulations that had P_{inf} <

Table 1 LRTs for top-down QMRAs

Study	Type	Virus	*Giardia*	*Crypto*	Bacteria	Notes
Barker *et al.* (2013)[38]	DPR	6.9	8		7.4	LRTs for municipal sewage scenario
	DPR	12.1	10.4		12.3	LRTs for outbreak conditions
Gerrity *et al.* (2023)[39]	DPR	13	10	10		Used 97.4th percentile pathogen concentrations and included a 10-fold safety factor for viable but nonculturable enterovirus; described tolerance to off-specification conditions rather than redundancy
	DPR	15	11	11		Used maximum pathogen concentrations; described tolerance to off-specification conditions rather than redundancy
MacNevin and Zornes (2020)[44]	DPR		5	5		Minimum LRTs for any WWTP
	DPR		9.5	10		Maximum LRTs for any WWTP
Page *et al.* (2015)[40]	General reuse	5.8	4.8	4.8	5.3	LRTs based on stormwater
Page *et al.* (2015, 2016)[42,43]	IPR	5.5	4.9	4.9	5.5	LRTs based on stormwater
Pecson *et al.* (2023)[19]	DPR	17	14	14		Included 4 log redundancy to protect against failures
Seis *et al.* (2020)[41]	IPR	<12				Compared different modeling approaches for concentration data: separate point estimate
	IPR	>16				Compared different modeling approaches for concentration data: separate modeling
Soller *et al.* (2018)[45]	DPR	14	12	12		95% of simulations have cumulative annual risks less than 10^{-4}
	DPR	15	13	13		100% of simulations have cumulative annual risks less than 10^{-4}
	DPR	16	11	11		100% of simulations have cumulative annual risks less than 10^{-4}
California Regulations[17,46]	DPR	20	14	15		Included 4 log redundancy to account for a 6 log undetected failure and used updated maximum point estimates
	IPR	12	10	10		Used maximum point estimates
Colorado Regulations[49]	DPR	12	10	10		Could be as low as 8/6/5.5 (virus/*Giardia*/*Crypto*) if justified by pathogen monitoring
Nevada Regulations[48]	IPR	12	10	10		
Texas Regulations[85]	DPR	8	6	5.5		Minimum LRTs, with actual LRTs potentially higher based on monitoring data; LRV calculation begins after WWTP
Florida Regulations[34]	IPR	14	12	12		

10^{-4}, while Gerrity *et al.*[39] calculated the required LRVs and then rounded to the nearest whole number, even if that meant rounding down. For LRVs of 15/11/11, Soller *et al.*[45] found that 99.7% of simulations met the probability of infection benchmark of 10^{-4}.

Gerrity *et al.*[39] summarized the regulations for IPR and DPR in the United States, in addition to performing bottom-up and top-down DPR QMRAs. For IPR, California requires LRVs of 12/10/10 for viruses, *Giardia*, and *Cryptosporidium*, respectively, although additional stipulations are required for surface water augmentation *vs.* groundwater replenishment. Colorado also implemented the 12/10/10 framework but for DPR,[49] and in Texas, where the LRV calculation begins in the treated wastewater effluent, minimum LRTs of 8/6/5.5 are required for DPR.[39,46,85] LRTs for DPR in Texas may be higher if warranted by the pathogen monitoring campaign required for each case-by-case DPR permit. For DPR, California targeted a 2.7×10^{-7} daily risk of infection benchmark, rather than an annual risk of 10^{-4}. While this does not impact point estimate QMRAs, it does impact the results for more complicated, Monte Carlo QMRAs by eliminating the aforementioned averaging effect in the annual risk calculation. California found baseline LRVs of 16/10/11 to be adequately protective of public health, and this determination assumed prior point estimate concentrations for *Giardia* and *Cryptosporidium*, a peak norovirus concentration reported in the literature,[63] and a daily ingestion volume of 2 L spread equally over 96 ingestion events per day.[39] However, California set its final LRTs at 20/14/15 to account for a 6 log treatment failure necessitating a 4 log treatment redundancy.[17]

Regulations are often developed using point estimates based on maximum concentrations, assumed GC : IU ratios of 1 when using molecular data (*e.g.*, norovirus), and in conjunction with conservative dose–response models. Care should be taken when using maxima, as these peak concentrations are often not comparable across studies. An alternative approach involves using percentiles based on Blom's equation,[82] for example, from which 95th or 97.4th percentile concentrations can be determined from individual studies (*e.g.*, a site-specific sampling campaign) or across multiple studies.[69] Choosing a single measured point also makes the final risk estimates more susceptible to potentially non-representative site-specific conditions,[86] or even error from laboratory analysis. An expert panel from the National Water Research Institute found that California's DPR regulations resulted in inherent conservatism of 9–11 logs, which could result in overdesigned and unsustainable potable water reuse systems.[39,80] In other words, overly conservative LRTs can increase capital and operations and maintenance costs, while potentially yielding no appreciable improvement in public health protection. These scenarios–

and their long-term implications–can be mitigated when using either distributions or percentile point estimates in a QMRA, rather than maximum values.

3.13 Other considerations

The results of QMRAs can be community-specific because different communities also have different reuse needs. For example, the QMRA by Church *et al.*[50] focused on reusing dishwashing water and proposed a risk benchmark that was specific to their military scenario. Kimbell *et al.*[34] performed a QMRA for water reuse at Zoo Miami, comparing different treatment trains. Although their exposure assessment was for humans ingesting 2.5 L d^{-1}, the recycled water will be used in the animal exhibits and is not intended for human consumption. They discussed how an interspecies QMRA would need to be done to evaluate the impact of recycled water on the most vulnerable species, which vary in size, habitat, physiology, water consumption, and metabolic rates.

Barker *et al.*[38] studied reuse in a small, remote community in Antarctica and compared municipal sewage pathogen loads with estimated loads during a gastroenteritis outbreak. They found that higher LRVs were needed in small communities to meet the benchmark of 10^{-6} DALYs due to the greater degree of contact between community members in a small population. If regulations are created from the pathogen levels in larger communities and applied to smaller communities with high contact, they might not be protective; conversely, LRTs developed for small communities may be overly stringent for large communities. Therefore, it is important to consider the local context before guidelines from one location are applied to another, highlighting the benefits of allowing tailored LRTs for different communities.

Commonly used risk benchmarks include 10^{-4} annual probability of infection pppy, as well as 10^{-6} annual DALYs pppy. However, it is possible to meet one of these benchmarks and not the other, depending on the severity of the disease. Lim *et al.*[27] found that risk from *Cryptosporidium* and norovirus were both mostly within the acceptable range of the WHO benchmark of 10^{-6} DALYs but consistently exceeded the 10^{-4} risk of infection benchmark. This highlights the need to determine what benchmarks are most relevant in a given context to protect public health without being unnecessarily stringent.

The focus of this review is the risk from microbial hazards, but depending on the level of treatment, there are also chemicals that could accumulate in potable reuse systems that could be harmful to public health, including heavy metals, disinfection byproducts, pharmaceuticals, and per- and polyfluoroalkyl substances (PFAS).[87,88] Keller *et al.*[89] conducted a review on technological, economic, and environmental considerations of DPR and included a partial list of chemicals of concern after advanced treatment. There could also be problems with public acceptance of potable reuse due to the so-called 'yuck factor'.[90]

Remy *et al.*[30] performed a life cycle assessment and a chemical risk assessment alongside their QMRA, which is important for understanding the cumulative health impact of recycled water. They found that while the proposed treatment would meet the 10^{-6} DALYs pppy target for pathogens, there would be an increase in constituents of emerging concern (CECs) in the IPR reservoir. Germany has health-based precautionary values for iopromide, iomeprol, gabapentin, and EDTA that Remy *et al.*[30] found could be exceeded in the reservoir. The concentrations of glyphosate and AMPA, a degradation product, would exceed the EU guidelines for pesticides (1 μg L^{-1} [91]), if they were applied to these chemicals.[30] Though this may be comparable to current wastewater treatment plants discharging to rivers without tertiary treatment, it highlights the importance of considering both chemical and microbial hazards. Their life cycle assessment found that IPR is competitive in terms of energy consumption and emissions with water importation and seasonal storage and is superior to seawater desalination. Kobayashi *et al.*[35] also performed a life cycle assessment, and highlighted how the local and global impacts of IPR differ. Reducing the local impact from pathogens resulted in a higher global 'cost' due to emissions leading to climate change. Page *et al.*[36] included other hazards to humans and the environment in their assessment, including nutrients and chemicals. They found that while the risks from organic chemicals were low, elevated iron levels exceeded potable water guidelines, if post recovery aeration was not employed. Dow *et al.*[92] found that while DPR could significantly reduce energy costs due to reduced pumping requirements from Lake Mead into the Las Vegas Valley, the net present value of DPR ranged from $1.0–4.0 billion, compared to $0.6 billion for the status quo IPR approach. The pairing of a life cycle assessment and chemical risk assessments to potable reuse QMRA would be a beneficial addition to future QMRAs.

4. Conclusions

This review included 30 publications that performed QMRA for potable reuse, encompassing case studies from Australia, France, Germany, Spain, the U.S., and Antarctica. The studies demonstrated that there are many factors that impact the risks estimated by potable reuse QMRAs, including the assumed ratio of gene copies to infectious units, the assumed volume and frequency of water ingestion, whether the simulation was dynamic or static, and if Bayesian modeling was used. Some decisions are often made for simplicity's sake (such as creating static, non-Bayesian models or assuming one ingestion event per day), but it is important for researchers to understand the basis and implications of these assumptions. Each QMRA is unique and will have different results because each audience/community has its own distinct context and needs. However, QMRAs should consider the impacts that critical assumptions such as ingestion frequency, pathogen concentrations, unit treatment processes, and treatment failures have on risk. The risk benchmarks (probability of infection or DALYs) are also location dependent and should be taken into consideration. This will allow the results to be better understood and contextualized.

As regulations are established and potable reuse becomes more widespread, it is crucial to protect human health without imposing excessively stringent requirements that are prohibitively expensive and do not necessarily enhance public health protection. One possible path forward is for regulations to become more flexible, such as was done in Colorado, where LRTs could be reduced if regular sampling provided sufficient evidence that human health would still be protected. By incorporating QMRA for potable reuse, LRTs could be developed for specific contexts, ensuring that health risks are accurately assessed and managed. Continuous monitoring and adaptive management strategies could be implemented to ensure ongoing compliance and safety, providing a dynamic response to emerging data and technological advancements. Due to the rise in wastewater surveillance for public health purposes, more robust and extensive pathogen datasets are expected to be published. Pathogen concentration variability and driving factors will be better characterized, which could reduce potentially unnecessary redundancies which have been built into QMRAs due to uncertainty. Implementing flexible regulations could promote the sustainable and safe expansion of potable reuse systems. Finally, recent publications demonstrate the value and importance of simultaneously evaluating microbial and chemical risks in the context of sustainability and life cycle assessment.

Abbreviations

DALY	Disability adjusted life year
NoV	Norovirus
AdV	Adenovirus
EnV	Enterovirus
Crypto	*Cryptosporidium*
Campy	*Campylobacter*
QMRA	Quantitative microbial risk assessment
DPR	Direct potable reuse
PCR	Polymerase chain reaction
RWC	Recycled water contribution
DFR	*De facto* reuse
IPR	Indirect potable reuse
SW	Surface water
GW	Groundwater
LRT	Log reduction target
P_{inf}	Probability of infection
LRV	Log reduction value
FAT	Full advanced treatment
DWTP	Drinking water treatment plant
CF	Cartridge filter
UV	Ultraviolet
MF	Microfiltration
UF	Ultrafiltration
NF	Nanofiltration
ESB	Engineered storage buffer
DW	Drinking water
pppy	Per person per year
TT	Treatment train
CBAT	Carbon-based advanced treatment
BNR	Biological nutrient removal
RO	Reverse osmosis
AOP	Advanced oxidation process
BAF	Biologically active filtration
WWTP	Wastewater treatment plant
WW	Wastewater
Cl_2	Chlorination
GC	Gene copies
IU	Infectious units
RBAT	Reverse-osmosis-based advanced treatment
MBR	Membrane bioreactor

Data availability

No primary research results, software or code have been included and no new data were generated or analyzed as part of this review.

Author contributions

Emily Clements—conceptualization, data curation, formal analysis, methodology, validation, visualization, writing – original draft, writing – reviewing and editing; Charlotte van der Nagel—data curation, formal analysis, writing – reviewing and editing; Katherine Crank—validation, visualization, writing – original draft, writing – reviewing and editing; Deena Hannoun—funding acquisition, supervision, writing – reviewing and editing, Daniel Gerrity—supervision, validation, writing – reviewing and editing.

Conflicts of interest

There are no conflicts of interest to declare.

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