Assessing Point of Use Water Treatment Technologies under Real-Use Conditions: The Field Challenge Test Technique

by

Camille Zimmer
B.Sc., University of Calgary, 2016
M.A.Sc., University of Victoria, 2019

A Dissertation Submitted in Partial Fulfillment of the Requirements for the Degree of

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in the Department of Civil Engineering

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University of Victoria

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Supervisory Committee

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Abstract

Point of use water treatment (POUWT) technologies can be the final and sometimes only barrier against waterborne illness in contexts where there is insufficient access to a safely managed on-premises water supply. Microbiological effectiveness of POUWT devices is currently evaluated under controlled laboratory conditions using water spiked with virus, bacteria, and/or protozoa or their surrogates to measure $\log_{10}$ reduction values or LRVs, in a process called challenge testing. However, laboratory-based POUWT challenge tests do not adequately assess microbe reduction under real-use conditions, thus omitting variations relative to factors such as user behaviours and water quality.

The overall aim of this work was to develop a method with which POUWT technologies can be evaluated under real-use conditions, which we refer to as the field challenge test technique. To this end, we validated the use of probiotic *Escherichia coli* (*E. coli* Nissle, EcN) and *S. cerevisiae* (baker's yeast) as field-appropriate, food-safe surrogates for pathogenic bacteria and protozoans, respectively. We implemented the innovative field challenge test technique using validated EcN and *S. cerevisiae* surrogates. In summer 2021, 144 one-on-one surveys were conducted of backcountry campers in the Juan de Fuca provincial park in British Columbia, Canada. The field challenge test consisted of spiking a 1 L sample of water with EcN and *S. cerevisiae* and requesting participants to treat the spiked water as they normally would, using their own POUWT device. Post-treatment water samples were enumerated in comparison to the original spike to calculate LRVs.

Using field challenge testing, we were able to ascertain the performance of POUWT methods under real-use conditions. Our field-based LRVs were generally lower than claimed by POUWT device manufacturers for the bacterial microbe class, but for the protozoan microbe class, LRVs were similar to those claimed by manufacturers. Using the framework of quantitative microbial risk assessment (QMRA), we quantified and compared health risk estimates when using laboratory-gathered vs field-gathered LRVs of POUWT devices. Health risks attributable to the bacterial pathogen class were higher based on field-gathered LRVs (i.e., obtained by field challenge testing) in comparison to corresponding manufacturer-claimed LRVs.For the protozoan pathogen class, calculated health risks were similar due to homogeneity between field-obtained and manufacturer-

claimed LRVs. The field challenge technique and corresponding QMRA analysis have numerous implications, including validation of POUWT sanitary inspection criteria, quantifying health impacts of contextual factors, or to inform technology selection.

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List of Acronyms, Abbreviations and Symbols

Acronyms and Abbreviations

AGI: Acute gastrointestinal illness

ANSI: American National Standards Institute

ANOVA: Analysis of variance

BPB: Butterfield's phosphate buffer

BSF: Biosand filter

CFU: Colony forming unit

CI: Confidence interval (i.e., 95% CI)

C. jejuni: *Campylobacter jejuni*, a pathogenic gram-negative bacteria

CONSORT: Consolidated standards of reporting trials

C. parvum: *Cryptosporidium parvum*, a protozoan pathogen

CT: Concentration-time (i.e., dose of chemical disinfection)

CWF: Ceramic water filter

DALY: Disability-adjusted life years

DBP: Disinfection by-product

DLS: Dynamic light scattering

DO: Dissolved oxygen

DOP: Demonstration of performance

DWT: Drinking water treatment

DWTP: Drinking water treatment plant

E. coli: Escherichia coli

EcN: *Escherichia coli* Nissle, a gram-negative bacteria

EcK12: *Escherichia coli* K-12, a gram-negative bacteria

ELISA: Enzyme-linked immunosorbent assay

FC: Free chlorine

FCR: Free chlorine residual

G. lamblia: *Giardia lamblia*, a protozoan pathogen

HACCP: Hazard analysis critical control point

HWTS: Household water treatment and storage

IT: Intensity-time (i.e., dose of UV disinfection)

JMP: Joint monitoring programme (i.e., of the WHO/UNICEF)

LAMP: Loop-mediated isothermal amplification

LDL: Lower detection limit

LDV: Laser doppler velocimetry

LMICs: Low- and middle-income countries

LP: Low pressure (i.e., LP-UV)

LRV: Log_{10} reduction value

LT2ESWTR: Long Term 2 Enhanced Surface Water Treatment Rule

MC: Monte Carlo simulation

MD: Mean difference (i.e., between two statistical groups)

MeSH: Medical subject headings

MICS: Multiple indicator cluster surveys

MPN: Most probable number

NCR: Normal counting range

NSF: National Sanitation Foundation (of the USA)

NTU: Nephelometric turbidity unit (i.e., of turbidity)

PCR: polymerase chain reaction

PDF: Probability density function

POU: Point of use

POUWT: Point of use water treatment

PRISMA: Preferred reporting items for systematic reviews and meta-analyses

PSD: Particle size distribution

QC: Quality control

QMRA: Quantitative microbial risk assessment

ROS: Reactive oxygen species

RT-PCR: Real-time polymerase chain reaction

S. cerevisiae: *Saccharomyces cerevisiae* (baker's yeast)

SDGs: Sustainable Development Goals

SODIS: Solar water disinfection

STROBE: Strengthening the reporting of observational studies in epidemiology

TSA: Tryptic soy agar

TSB: Tryptic soy broth

UNICEF: United Nations International Children's Fund

USEPA: United States Environmental Protection Agency

UV: Ultraviolet radiation

UVA_{254}: UV absorbance of water across a 1 cm path length at 254 nm wavelength

UV-LED: Ultraviolet light emitting diode

VBNC: Viable but non-culturable cells

VLPs: Virus-like particles

WHO: World Health Organization

YEPD: Yeast extract peptone dextrose broth

Y&M-CI: Yeast and Mold Color Indicator method

ZP: Zeta potential

Symbols

α: Shape parameter for the Beta-Poisson approximation (unitless)

$\boldsymbol{b}$: Lag coefficient for dose-response of a microorganism to disinfection

$\boldsymbol{C_{ND}}$: Non-detect pathogen concentration in raw water (CFU or oocysts per L)

$\boldsymbol{C_{raw}}$: Raw water pathogen concentration (CFU or oocysts per L)

$\boldsymbol{D_{refill\,n}}$: Dose per n^{th} refill (CFU or oocysts per refill)

$\boldsymbol{D_{trip}}$: Dose per 7-day backpacking trip (CFU or oocysts per trip)

$\boldsymbol{\Delta D_{trip}}$: Difference in dose (# per trip)

$\boldsymbol{DALY_{trip}}$: DALYs per 7-day backpacking trip (DALYs per trip)

$\boldsymbol{\Delta DALY_{trip}}$: Difference in $DALY_{trip}$ (DALYs per trip)

DW: DALY weighting (DALYs per illness)

k: Inactivation rate constant for dose-response of a microorganism to disinfection

$\boldsymbol{N}$: Number of microorganisms after water treatment (unitless)

$\boldsymbol{N_0}$: Number of microorganisms before water treatment (unitless)

$\boldsymbol{N_{50}}$: Median infectious pathogen dose (unitless)

$\boldsymbol{P_{ill \mid inf}}$: Probability of illness given infection (unitless)

$\boldsymbol{P_{ill,trip}}$: Probability of illness per 7-day backpacking trip (unitless)

$\boldsymbol{P_{inf,refill\,n}}$: Probability of infection per n^{th} refill (unitless)

$\boldsymbol{P_{inf,trip}}$: Probability of infection per 7-day backpacking trip (unitless)

$\boldsymbol{\Delta P_{inf}}$: Difference in P_{inf} (unitless)

PP: Pathogen prevalence (i.e., the probability that any given water refill is contaminated with the organism of interest, %)

r: Shape parameter for the exponential function

$\boldsymbol{S}$: Susceptible fraction of the population (%)

μ: Group mean

σ: Group standard deviation

V: Drinking water consumption per refill (L)

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Alice Gentleman and Peanut the dog, Sombrio Beach, May 2021.

Dedication

To Marjorie Zimmer (April 5, 1935 – October 5, 2022)

and Glenn DiGeorgio (September 2, 1936 – October 25, 2022)

Tas far

Chapter 1: Introduction

1.1 Motivations

Clean and safe drinking water is fundamental human right and basic tenet of human dignity[1], and is a key component of sustainable and inclusive development, as recognized by the Sustainable Development Goal 6 (SDG 6: Clean Water and Sanitation)[2]. Lack of clean water is a primary determinant of perpetuating poverty (SDG 1: No Poverty)[3,4], while its provision is a proven way to ensure good health and well being (SDG 3: Ensure Healthy Lives)[5]. Children tend to manifest the most severe health impacts of unsafe drinking water[6], which often translates into poor nutritional status (SDG 2: Zero Hunger)[7] and compromised school performance (SDG 4: Quality Education)[8]. Women typically occupy primary roles to collect, store and treat water for their households and therefore face a host of specific challenges and risks regarding (un)clean drinking water[9], which have intersectional impacts regarding empowerment and equality (SDG 5: Gender Equality)[10,11]. Access to safe water has other important quality of life benefits such as reduced stress and increased time savings, well-being, and security[12].

Despite the importance of clean water, more than 2 billion people globally lack access to safe and effectively treated water[13]. Microbiologically contaminated drinking water can transmit diarrhoeal disease, which is estimated to cause 485,000 deaths annually[14]. SDG target 6.1 aims to "achieve universal and equitable access to safe and affordable drinking water for all" by the year 2030[2]. Where this goal has not yet been reached, such as low-resource contexts or during emergency or humanitarian crises, water can be treated in the home by point-of-use water treatment (POUWT) technologies, which are currently used by over 1 billion people globally as the final (and sometimes only) barrier against waterborne illness[15]. There is a wide range of options for POUWT methods available, such as chemical disinfection (e.g., chlorine tablets), UV disinfection (e.g., sunlight) or filtration (e.g., ceramic filter). Regardless of the context or choice of product, most POUWT

techniques rely on common treatment principles (i.e., chemical disinfection, UV disinfection and/or size exclusion). Health risks due to unclean drinking water are expected to increase as water quality degrades with climate change[16,17], making treatment imperative.

How well do POUWT technologies "work"? This question can be answered using a process called *challenge testing* (also referred to as *microbiological testing* or *efficacy testing*; Figure 1.1) to look at the microbiological performance of POUWT methods. Challenge testing consists of spiking water with virus, bacteria, and/or protozoa or their surrogates and treating water to determine the efficacy (log_{10} reduction values, LRV's, of pathogens or their surrogates; Equation 1.1) in a controlled laboratory setting[18,19]. This method of laboratory-based challenge testing likely overestimates LRVs (how well POUWT methods "work") and thus overestimates the level of health protection by POUWT[19,20]. Underperformance of POUWT in the hands of end-users *in situ* (as compared to the laboratory) could be due to reasons including user behaviour or non-compliance, variable-quality source water, or cross-contamination during treatment[21–29].

Equation 1.1 Calculation of the log reduction value (LRV), given the number of microbes before (N_0) and after (N) water treatment.

$$LRV = log_{10}\left({}^{N}/_{N_0}\right)$$

Why does this overestimation matter? POUWT efficacy data is used to conduct risk assessments via a framework called quantitative microbial risk assessment (QMRA). Such QMRA assessments can be used to estimate expected health gains from introducing a given POUWT method into a community[30] or to examine the trade-off between POUWT efficacy and adoption[31–35]. Based on QMRA assessments, recommendations are made by public health organizations or local governments regarding method selection or guidance for treatment. Therefore, if the performance (LRVs) of POUWT methods has been overestimated using laboratory-based data, and end-users are inconsistently or improperly using such methods resulting in decreased performance, then such public health recommendations could be inaccurate, leaving room for improved recommendations based on more reflective data[20].

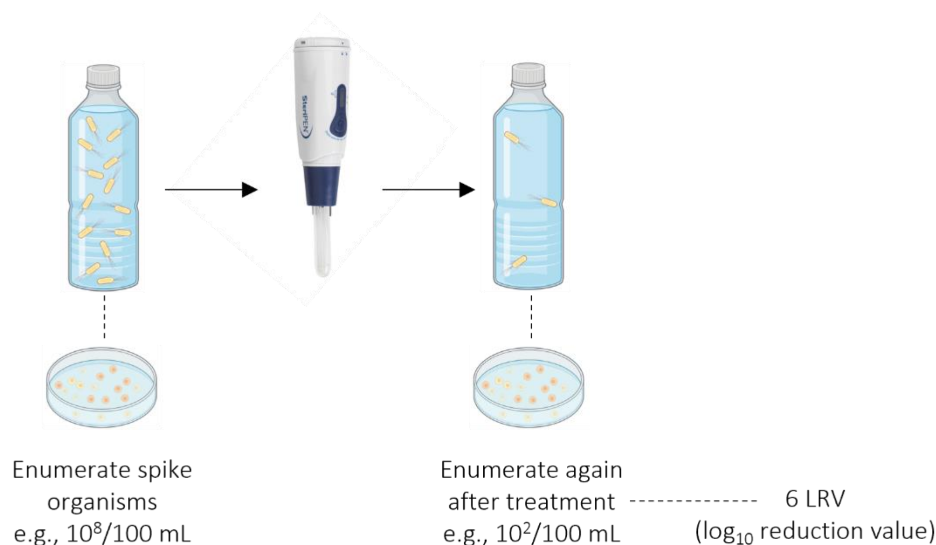

Figure 1.1: Schematic of one microbiological challenge test with a Steri-Pen (UV disinfection).

Ideally, POUWT methods would be challenge tested *in situ*, as opposed to the laboratory, to obtain more reflective data of the end user experience[20]. The main barrier towards carrying out such *in situ* performance evaluations is a lack of appropriate spike organisms required for challenge testing, particularly in low-resource contexts. The spike bacteria, virus or protozoa must be safe for human consumption (i.e., "food-safe") to be used outside a laboratory setting and to test POUWT techniques under actual use, to satisfy ethical and safety requirements for human study participants[20]. In addition, spike organisms should be easily transportable and culturable using feasible techniques that can be deployed outside the laboratory setting. An appropriate bacterial surrogate has been identified and was subject to preliminary validation through previous work[36], although not yet validated for challenge testing. Baker's yeast (*Saccharomyces cerevisiae, S. cerevisiae*) has been identified as a possible non-pathogenic surrogate for protozoans in application to filtration methods[37,38], has been applied as a challenge organism to evaluate *in situ* microbiological performance in non-potable water applications[39,40] and has been recommended for further use for other *in-situ* evaluations[41], although has not yet been validated as a protozoan surrogate for water disinfection applications. If field-appropriate surrogate microbes were identified and validated, the methodology of field challenge testing would then need to be established and piloted. These research gaps form the basis for the project objectives (discussed in Section 1.2; dissertation structure also outlined below).

1.2 Project Aim and Objectives

The overall aim of this work was to develop a method under which POUWT technologies can be evaluated under real-use conditions (also referred to as *in situ* or field conditions). This aim was addressed via four specific objectives, described below.

1.2.1 Specific Objective 1 (SO1)

The first objective was to examine the evidence of a discrepancy between laboratory and field assessments of POUWT methods, and to summarise any underlying factors for compromised POUWT field performance. Although some studies have directly compared POUWT performance in the laboratory and field contexts[19,21,22,25–29,42], there remains a gap in collating and evaluating such studies as a whole.

1.2.2 Specific Objective 2 (SO2)

The second objective was to identify and validate appropriate bacterial and protozoan spike microorganisms for use in evaluating POUWT methods *in situ*. As outlined above, the spike microbes under study were a probiotic *E. coli* and *S. cerevisiae* as surrogates for pathogenic bacteria and protozoans, respectively.

1.2.3 Specific Objective 3 (SO3)

This objective was to develop and implement *in situ* challenge test protocols of POUWT methods being used in the backcountry using the food-safe bacterial and protozoan surrogates described above. The goal was to ascertain the performance of such POUWT methods during regular use and develop the methodology of field challenge testing, which is novel.

1.2.4 Specific Objective 4 (SO4)

The fourth and final objective was to contextualize the results of the novel fieldwork programme by conducting a QMRA analysis. The goal was to compare estimated health impacts of using laboratory-based vs field-obtained LRV data on health risk assessments and associated recommendations. Thus, we intended to provide a framework within which to contextualize field-based LRV data against health risk benchmarks, disaggregated by choice of treatment method (i.e., chemical or UV disinfection, or filtration).

1.3 Background

1.3.1 Project context

The work detailed in this dissertation commenced in September 2019 and was thus considerably disrupted by the COVID-19 pandemic, which began to impact Canada in March 2020. Although critically necessary to save lives, limitations on in-person interactions, stay-at-home orders and international border lockdowns were in place for much of the duration of my PhD, particularly during the critical project planning phase. There was also a high degree of uncertainty regarding the timing and extent of COVID-19 restrictions, adding to difficulties to plan and execute any proposed fieldwork.

Although POUWT methods are widely used in many low- and middle-income countries (LMICs)[43], it was neither feasible nor ethical at the time of this work to visit low-resource settings in which POUWT technologies are generally used. Instead of looking to do fieldwork internationally or in low-resource communities within Canada during a global pandemic, we opted to study POUWT methods in the context of backcountry campers, who are generally relatively healthy individuals voluntarily undertaking safety and health risks for a limited time. The outdoor nature of backcountry camping was compatible with COVID-19 safety protocols.

POUWT methods are commonly used during backcountry recreational activities, such as camping or backpacking, where hikers and campers can be exposed to waterborne enteric pathogens such as *Giardia lamblia* (*G. lamblia*), *Cryptosporidium parvum* (*C. parvum*)[44] and/or pathogenic strains of *Escherichia coli* (*E. coli*)[45]. Long-distance backpackers have reported diarrhea as one of the most common medical complaints, along with dermal and musculoskeletal injuries[46,47], often resulting in lost hiking days and/or early trail exit[47]. Diarrhea is the most prevalent illness among backpackers[46,47], with estimated incidences during backcountry trips varying from 3-5%[48] to 56%[46]. Although the treatment mechanisms of POUWT methods are similar between backpacking and LMICs, the context of backcountry recreants is not similar to the context of the 2 billion people globally who do not have access to safe and effectively treated water[13]. Positioning our fieldwork in the space of backcountry camping does not equate the difficulties faced by those unserved by SDG 6 with otherwise healthy backpackers who may incur injury or illness while voluntarily undertaking a risky activity.

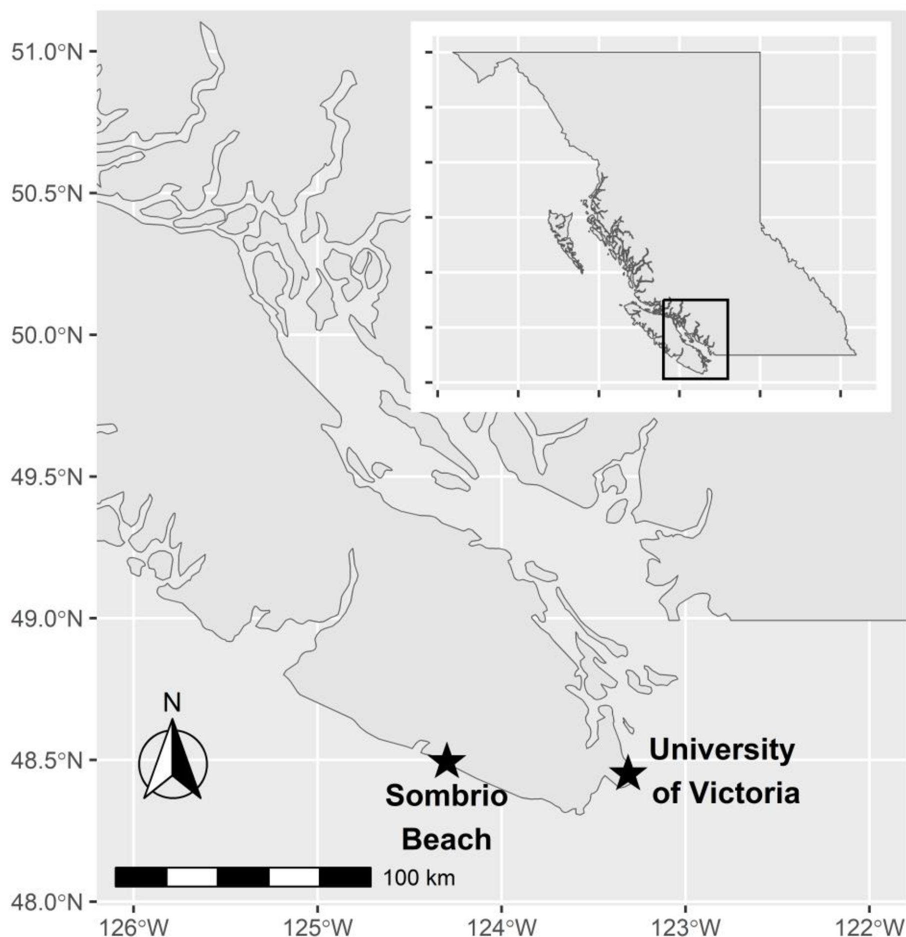

Figure 1.2: Map of British Columbia, Canada (inset), with Sombrio Beach and the University of Victoria labelled.

Due to restrictions related to COVID-19, fieldwork related to SO3 and SO4 was conducted in Summer 2021 at the Sombrio Beach campsite, in the Juan de Fuca Provincial Park in British Columbia (BC), Canada, on the west coast of Vancouver Island (Figure 1.2). Sombrio Beach is a campground that is located on the traditional territories of the Pacheedaht First Nation[49]. The site is accessible via car and is an entry/exit point located approximately halfway through the Juan de Fuca Marine Trail, a 47-km coastal backcountry hiking trail[50]. The beach is therefore popular with two groups of campers: either car campers or backpackers hiking through the site. Backpackers hiking through Sombrio Beach were the main target participants of our work, as there is no source of treated drinking water at Sombrio Beach, and backpackers must treat their own water for drinking using POUWT methods (campers arriving via car generally bring treated water from home). The main water source at the site was Sombrio Creek, a freshwater creek

with headwaters in the Juan de Fuca Park and nearby logging areas. During the summer of 2021, we interviewed campers on Sombrio Beach who were using POUWT devices to treat their water for drinking. The interviews included a questionnaire, a grab sample of the participants' personal drinking water, a hand rinse test (i.e., measuring the bacterial contamination of participants' hands) and a field challenge test. The fieldwork received ethical approval from the University of Victoria (#21-0092), authorization from BC Parks (#98700-20), and is further explained in Chapter 6.

1.3.2 Background on point of use drinking water treatment

Filtration

Filtration is a physical treatment process that is widely used to remove particles (including pathogens) from water. It is defined as any process that removes solid particles from a suspension by passage through a porous medium[51] and takes many forms that can be divided into two broad categories[19,52].

The first category of filtration is granular filtration, which uses a combination of removal mechanisms: surface straining, when particles larger than void spaces are retained at the surface; cake filtration; where particles are retained by a cake of larger material that collects at the filter surface; and depth filtration, where particles are removed throughout the filter by attachment to filter grains[51]. Granular filtration approaches in application to POUWT include rapid granular media filters and biosand filters (BSF)[19,42]. Rapid granular media filters are typically comprised of sand or successive layers of anthracite coal and sand inside of a bucket, barrel or drum, and utilize depth filtration as the dominant mechanism of removal, with water flowing through the media as it is added[51]. They are widely used, being simple, low-cost and potentially efficacious at pathogen removal[19,42]. BSFs, also referred to household slow sand filters, are generally larger in size than rapid granular media filters, and are comprised of large barrels or a purpose-built concrete structure containing a sand bed of approximately 1 m height supported by a gravel underdrain system[42,53]. The filters rely on surface straining and cake filtration as the dominant mechanisms of removal and are intended to operate with a constant head, which fosters the growth of the "schmutzdecke" slime layer, additionally promoting pathogen removal by biological processes such as predation[42,51,53]. BSFs are relatively simple to build and operate, but head must be maintained to prevent the slime layer from drying and to produce an adequate volume of water for a household, as filtration takes place slowly[42,51,53].

Microbial reductions by BSFs can be high if filters are adequately constructed and maintained, however this is difficult to achieve in practice[42].

The second main category of POUWT filtration consists of membrane filters and ceramic water filters (CWFs)[19], which operate mainly by size exclusion and cake filtration[19,51], although CWFs can achieve particle adhesion within the ceramic matrix under certain conditions[54]. Porous CWFs are comprised of a ceramic filter element (disk, tube, candle or pot) suspended within a covered reservoir having a 10 – 20 L capacity. Ceramic water filters rank highly in terms of effectiveness, cost, ease of use and microbiological protection[54,55]. Membrane filters include filters made of cloth or other woven fabrics, which give generally poor microbiological protection[19], as well as micro- or ultra-filtration methods[56], which tend to be purpose-made and therefore higher-cost but give higher pathogen removal that varies with pore size[19].

Each approach to filtration has its own advantages and disadvantages as briefly described above but share some common features. In principle, filters should provide clean water over a relatively long life, with little to no maintenance costs following the initial outlay to purchase or make the filter[52]. With the exception of micro- or nanofiltration, filters are generally locally produced using locally-available materials which makes production robust to supply chain disruptions[52,53,57–59]. Filters generally have high user acceptability due to the ease of use and improved look and taste of the water, and are proven to effectively remove protozoan and bacterial pathogens[19,52]. However, they are not typically effective at removing viral pathogens, and they do not provide residual protection, opening the possibility of water recontamination following treatment[19,52]. Filter maintenance can jeopardize the potential for microbe removal if carried out incorrectly and there is a need to educate the user on these requirements if health impacts are to be realized[52].

Chlorine disinfection

Chlorine disinfection typically consists of treating water with dilute sodium hypochlorite (NaOCl) with the goal to produce hypochlorous acid (HOCl; favoured at a pH of 7.5 or lower), a strong reducing agent with biocidal activity[51]. Chlorine disinfection is effective to reduce bacteria and most viruses[52] and provides a free chlorine residual (FCR) that protects water against recontamination for a limited period of time[60].

There is a body of evidence supporting point-of-use chlorination to effectively improve water quality in development contexts[61], although recent literature suggests that a reliance

on individual adoption for effective treatment over an extended period of time may limit widespread public health benefits due to challenges of sustained, consistent and correct use[62]. Passive inline chlorination has also been adopted in low-resource contexts to treat water and provide a FCR at the water source (e.g., at a borehole, water point or pre-storage tank), eliminating the requirement of individual behaviour change[63].

Chlorine is widely used to provide treated water during a humanitarian emergency response[64]. There are a wide range of approaches using myriad products and at various points of addition, such as the point of delivery (e.g., in-line at a tap stand or water truck), point of source (e.g., chlorine dispensers) or point of use (e.g., household distribution)[64].

Although there are demonstrable benefits of using chlorination to treat water in low-resource contexts[52,61,65], drawbacks include difficulties with sustained adoption as mentioned above[62], relatively low protection against some viral pathogens and protozoan parasites, especially *Cryptosporidium parvum* (*C. parvum*), and potential user objections due to the taste and odour of chlorinated water[52]. Because chlorine is a strong reducing agent, it will react indiscriminately with most organic and inorganic materials, including pathogens, metals, humic and fulvic acids[51], with two possible negative outcomes. First, the reacted chlorine becomes unavailable for further disinfection, and the amount of chlorine used up in these reactions is called the "chlorine demand". If the chlorine demand exceeds the concentration of FC added to water, usually due to a high organic or inorganic content, then there will not be any chlorine remaining to continue disinfection and/or provide a FCR, limiting effectiveness[51]. Second, the reaction of FC with humic and fulvic acids in water produce disinfection by-products (DBPs), which are known to be carcinogenic if consumed over an extended period of time (i.e., chronic exposure)[51,64]. Therefore, chlorination is the most effective when used in water with a low content of organic and inorganic components, a challenge in areas where choice of source water may be limited.

UV disinfection

UV disinfection in low-resource contexts typically takes the form of solar water disinfection (SODIS) or UV light-emitting diode technologies (UV-LEDs)[19]. UV disinfection has a proven health impact, providing reduction of key bacterial, viral and protozoan pathogens while leaving water taste unaffected, leading to high user acceptability[52]. Key wavelength ranges of the UV spectrum for disinfection are UV-A (400-315 nm) and UV-C (280-100

nm), which target cellular DNA to inactivate the microorganisms, rendering them unable to reproduce and therefore infect a new host[51].

The SODIS technique consists of placing water into transparent containers (usually 2 L PolyEthylene Terephthalate, or PET bottles) which are then exposed to the sun for 6 to 48 hours depending on sunlight intensity[66]. SODIS is a simple, accessible, low-cost treatment method that is independent of supply chains owing to the simple requirement of a clear 2 L water bottle[66]. Water recontamination is usually unlikely following SODIS because water is typically consumed directly from the small, narrow-mouthed bottles in which they are treated[52]. These factors lend SODIS its niche in the suite of available PoU water treatment technologies, however drawbacks of SODIS include the need to pre-treat the water that appears dirty in order to prevent "shielding" of pathogens by suspended particles in the water; limitations on the volume of water that can be treated at one time and the length of time required for treatment; the need for sustained, consistent behaviour change for realization of full health benefits[52], and the potential for microbes to regrow in the dark while the water is stored[67].

UV-LEDs have been identified as potentially suitable for low-resource contexts because they are small and robust with a long lifespan and low energy requirements[68]. They are also potentially suitable for areas in high-income countries that lack sufficient water treatment and distribution infrastructure (e.g., rural areas) because of their small size, energy efficiency and potential to be installed in-line with existing piped water supplies[69]. However, UV-LED technology for household and/or POUWT is at an early stage in development and technology challenges must be overcome in order to scale up LED-based water disinfection devices[69,70].

1.4 Dissertation Outline

This dissertation was written to be manuscript-based, such that each manuscript (chapter) is prepared with the intention to submit (or has already been submitted) to a peer-reviewed journal. There are five manuscripts, each addressing specific objective(s) as detailed in Table 1.1. Cover pages at the beginning of each chapter denote the publication status and authorship; author contributions are described at the end of each chapter. Following the five manuscripts, there is an "overall discussion" section (Chapter 7), where the broader implications of my PhD work are examined, with limitations and potential future research arising out of this project. In some sections of the dissertation, references are made to work "we" did because much of the work was distributed, although I did have a primary leading role in all the chapters detailed below.

Table 1.1: Outline of the five manuscripts comprising this PhD dissertation, mapped to project SOs.

Chapter number	Manuscript Title	SO addressed
2	Differences in laboratory versus field treatment performance of point-of-use drinking water treatment methods: Research gaps and ways forward	SO1
3	Validation of EcN as a Suitable Surrogate for EcK12 in Field Evaluation of Disinfection-Based Point-of-Use Water Treatment	SO2
4	Assessing the suitability of probiotic supplement EcN for microbiological quality control of ceramic water filters	SO2
5	*Saccharomyces cerevisiae* as a Protozoan Surrogate: Systematic Review and Meta-Analysis of Disinfection Methods in Drinking Water Treatment	SO2
6	Quantifying and comparing health risk estimates: laboratory vs field performance of POUWT devices in a real-world setting	SO3 & SO4

Chapter 2: Differences in laboratory versus field performance of point-of-use drinking water treatment methods: Research gaps and ways forward

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2.1 Abstract

In this Perspective, we present evidence that indicates a discrepancy between laboratory and field performance of point of use water treatment (POUWT) techniques, identified via a narrative review process to investigate the origin of the LRV comparison estimates reported by the WHO. We considered only peer-reviewed articles that reported laboratory and field log reduction values (LRVs) for the same POU technology. We will present a summary of explanations that have been offered by the literature regarding such discrepancies; the potential implications of the "laboratory versus field" data discrepancy; and potential risks posed by conflating the two. Finally, in view of this discussion, we propose a strategy to help mitigate the research gap and explore the potential to improve current health risk assessments and ultimately, recommendations by public health entities and manufacturers of POUWT products.

2.2 Introduction

Clean drinking water is a basic human right[71]; however, access to safe drinking water is not universal. Globally, an estimated 1.8 billion people drink water that is contaminated with faecal indicator bacteria thermotolerant coliform or *Escherichia coli*[72] and are thus at risk of diarrhoeal diseases[73]. Especially at risk are children under 5 years of age, for whom diarrhoea is the fifth leading cause of death[74], with unsafe water and unsafe sanitation accounting for 72% and 56% of diarrhoea deaths, respectively[74].

The UN's Sustainable Development Goals (SDGs) 6.1 was set to "achieve universal and equitable access to safe and affordable drinking water for all"[75]; in order to sustainably address this goal, safe drinking water should be viewed as a "source to sip" framework[15] (Figure 2.1). Water should be collected from an improved source[76] that is accessible, sustainable, and of adequate quality[77]; transported using a clean fetching container[78,79]; treated consistently and correctly over a sustained period[31–34,80] using a device that has been adequately operated[21,25,81–84]; stored using a clean vessel after treatment[78,79,85], and consumed using a clean cup[24,78,86]. Taken together, these important components comprise household water treatment and safe storage (HWTS), which can be employed to provide protection against diarrheal illness[19], potentially resulting in substantial positive health impacts[87]. Use of HWTS is widespread: an estimated 1.1 billion people employ HWTS practices[43], and in contexts where SDG goal 6.1 has not yet been reached and there is insufficient or non-existent access to a safely managed on-premises water supply, HWTS is key to protecting public health.

Although all links in the "source to sip" chain as depicted in Figure 2.1 are important to protect consumer health, this Perspective focuses on point-of-use water treatment (POUWT) methods, which are the final – and sometimes only – safety barrier against waterborne disease. There is a wide range of POUWT techniques, most commonly taking the form of chemical disinfection (e.g., hypochlorite), UV disinfection (e.g., solar disinfection) or filtration (e.g., ceramic filtration)[88].

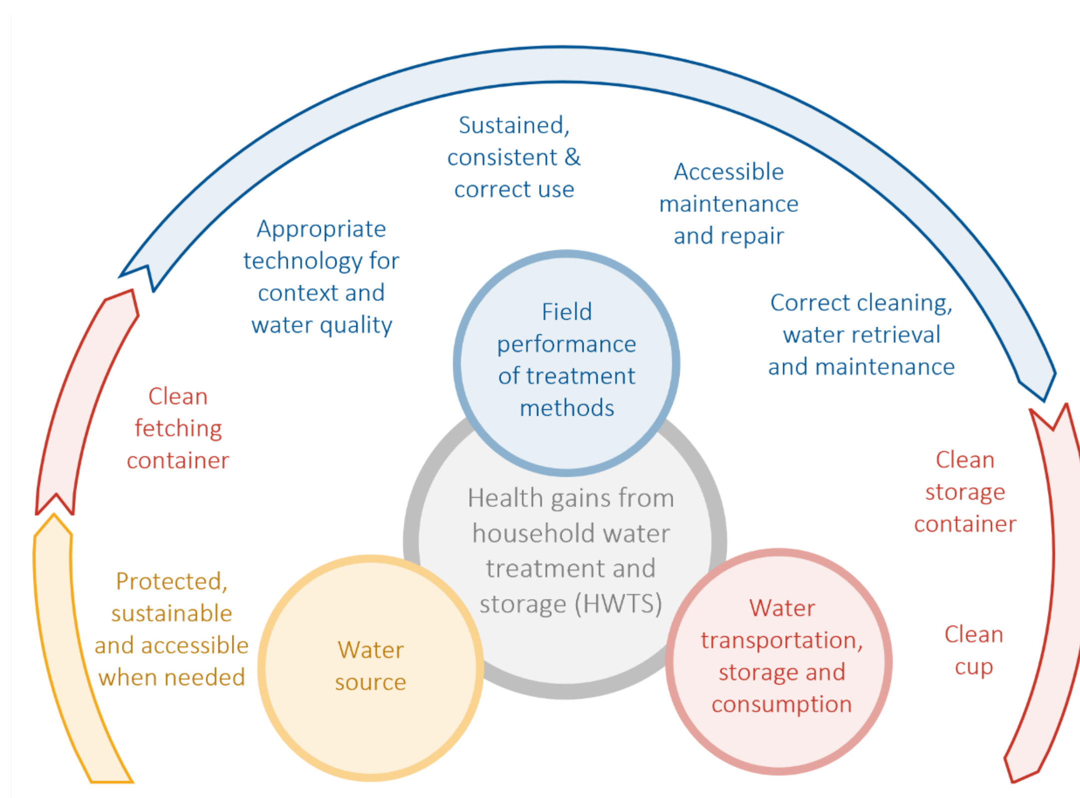

Figure 2.1: An overview of the "source to sip" framework[15], adapted for HWTS. POUWT methods are one part of effective HWTS. Field performance of POUWT methods, as well as factors affecting such, are the focus of the present Perspective.

"How well do POUWT methods reduce waterborne microbial risks?" This question can be answered using a process called challenge testing (also referred to as microbiological testing or efficacy testing) to look at the microbiological performance of POUWT strategies. Challenge testing consists of spiking test water with virus, bacteria, and/or protozoa or their surrogates and treating water to determine the microbiological reduction efficacy ($\log_{10}$ reduction values, LRVs, of pathogens or their surrogates) in a controlled laboratory setting[18,19]. Although laboratory-based challenge testing is a valuable tool to evaluate the performance of POUWT approaches under controlled and replicable conditions, we posit that such a controlled environment represents a "best-case scenario" – even if test conditions are intended to mimic poor source water quality – and challenge testing potentially does not provide an accurate representation of POUWT performance experienced by the end user.

Laboratory-generated POUWT efficacy data is contextualized via health risk assessments using the quantitative microbial risk assessment (QMRA) framework. QMRA can be used

to estimate expected health gains from introducing a given POUWT method into a community[30,89] or examine the trade-off between POUWT efficacy and compliance during use[31–34,80]. We will show in this Perspective that such QMRA analyses are utilizing performance data (LRVs) of POUWT methods that have been overestimated using laboratory-based studies; consequently, the conclusions reached by such QMRA analyses could be inaccurate. There is a research gap to gather more representative data (i.e., field-based assessments); such techniques do exist[36–41] but are not directly applicable to low-resource contexts.

The objective of this Perspective is to explore the data discrepancy between laboratory and field assessments of POUWT methods. We will present the evidence of such a data discrepancy, discuss the resultant public health implications, and propose a strategy to fill this space.

2.3 POUWT performance in the laboratory and field

2.3.1 Methodology

Our narrative review grew from the impetus to investigate the origin of the LRV comparison estimates (i.e., laboratory-based LRVs, or best-case scenario, versus field-based LRVs, or baseline performance) published by the WHO[19,42], which were not systematically derived. The studies we examined for this Perspective were identified in a narrative process via two avenues: 1) by investigating papers cited by the WHO[19,42] to construct LRV comparison estimates (i.e., laboratory-based LRVs, or best-case scenario, versus field-based LRVs, or baseline performance), which were not systematically derived; and 2) by searching scholarly databases (e.g., Google Scholar, Web of Science) using search terms similar to those in studies referenced by the WHO[19] (e.g., "Ceramic filt*" + "challenge" + "drink* water"). We included only peer-reviewed studies that directly reported laboratory and field log reduction values (LRVs) for the same POU technology.

2.3.2 The laboratory versus field performance discrepancy

Laboratory challenge tests are a useful tool that can tell us the likely maximum performance of the POUWT method under evaluation. This applies even if laboratory

assessments are intended to simulate challenging conditions, for example the use of a high-turbidity, high-organic-content test water[18,19,90]. Laboratory assessments can be effectively employed to identify water quality- or treatment-related limitations of products and screen performance between several design conditions[91,92], options or products[93,94].

In the field, microbiological performance of POUWT strategies is typically assessed by sampling water before and/or after treatment[93] (e.g., at the inlet and outlet of a filter), which is well-suited to evaluate compliance with health-based water quality targets. To a more limited extent, field evaluations can examine risk reduction or potential for protection offered to the end user by a given POUWT method, although such studies can be censured by influent microbe concentrations.

The data in Table 2.1 show that reported discrepancies range between 0.1 LRV[28] and 8 LRV[25]. Aggregate estimates published by the WHO[19] of the laboratory versus field performance discrepancy range between 1 and 4 LRV for virus, bacteria and protozoans. Field studies conducted on ceramic filters exemplify evidence of wide variation (i.e., several LRV) in the field data, between households and visits[21,84], and in the laboratory data, between filters and within individual filters over time[84]. In general, there was a paucity of direct comparisons between laboratory efficacy and field performance, especially with respect to solar disinfection (SODIS), for which no direct comparisons could be found. Comparative evaluations, although imperfect, provide important context-specific information and more such studies are needed.

Although not presented in Table 2.1, some studies noted a decline in other non-microbiological performance indicators between laboratory and field, such as decreasing ceramic filter flow rates over time[25]. This was especially noted in the case of high-turbidity source water[25] and/or elevated turbidity in filter effluent[24,95] (see Supplementary Table S2.2 through Table S2.6).

There are general limitations of comparing laboratory and field studies. Field studies typically report on bacterial reduction, excluding virus and protozoan reductions due to limitations in field quantification methods. Field study sampling points varied considerably. "Before treatment" water samples were collected from the water source[24,81,82,96,97] (e.g., local tap, borehole or surface water), stored water in the household[28] or directly from the top bucket of a filter[21,23,25]. "After treatment" water samples were collected directly from the bottom bucket of the filter[21] (bypassing the spigot), from the

filter spigot[25,28,82,97,98] (bypassing the drinking cup) or from the drinking vessel[81,97], possibly confounding treatment performance with potential re-contamination or re-growth. Variable environmental bacterial concentrations were noted as potentially driving variations in measured POUWT performance[24,28,99]. There is a relatively high potential for field POUWT performance to be censored or limited by environmental bacterial concentrations[21,84], which are typically several orders of magnitude lower than those used in spiked laboratory studies (which are not intended to simulate bacterial concentrations of natural waters), although censored data does occur in laboratory studies[25].

2.3.3 The laboratory versus field performance discrepancy, explained

Explanatory factors have been suggested regarding the observed discrepancy between laboratory and field data (Table 2.1). With respect to ceramic filters, inconsistent filter performance was sometimes due to varied manufacturing processes[82] and cracking or damage was cited as allowing short-circuiting of water through filter elements[24,25,100,101]. Decreased flow rates or blockage has been in some cases attributed to irreversible fouling including biofouling[25,101]. It was noted that lack of access to local supply chains for repair or replacement of such damaged filters hindered the performance[83,102]. Improper user cleaning practices, including backwashing or washing with unclean water[25,82,83] or touching hands to the external filter element or clean water receptacle[21,82,84] were observed, as were general user practices such as improper retrieval of water from filter (e.g., dipping hands into receptacle)[21] or using untreated water to rinse the drinking cup[83].

With respect to biosand filters, variable or unfavourable filter use conditions were postulated to explain the discrepancy between laboratory and field performance (e.g., frequency of use, treated water volume, residence/standing time of water within the filter and/or receptacle)[24,28]. For both ceramic and biosand filters, variable or poor source water quality (i.e., microbiological or non-microbiological quality) was cited as hindering microbiological performance[24,28].

Water quality was also cited as hindering chlorine disinfection due to the potential for free chlorine consumption to leave a decreased residual for disinfection[24,81], particularly in cases where no prior treatment occurred to remove turbidity or organic material prior to chlorination[81]. Similarly, natural variations in field water chemistry that were not present in the laboratory were cited for electrolytic disinfection with silver[103]. Long storage periods

or re-contamination of household storage containers can also consume free chlorine residual, leading to a decrease in disinfection and therefore microbiological reduction[81]. Incorrect or inconsistent chlorine dosage was also noted in the field, particularly in cases where procurement of chlorine is difficult or relatively expensive, where users sometimes aim to make supplies last longer by under-dosing their water[81]. In the case of electrochlorinator devices, running out of battery charge, breakage or technical problems caused a decline in performance and/or cease in use[24]. Variability of human use and unpredictable human factors were cited with respect to the laboratory versus field LRV discrepancy for silver electrolysis disinfection[103].

2.4 Why does the laboratory versus field performance discrepancy matter?

There are several potential implications of conflating laboratory-demonstrated microbiological efficacy with field-validated performance of POUWT techniques. Literature has been published[56] that reaches conclusions and recommendations based solely on laboratory-based data, ignoring factors impacting field performance and therefore potential end-user health protection. Although not applicable to most POUWT manufacturers, achievement of high LRVs in the laboratory could potentially give manufacturers license to imply that their devices confer a high degree of protection to the user, despite the fact that sustained, proper use may be difficult, as has been observed for some devices[25,83].

Some reported differences in laboratory versus field data[24,25,81,96] (Table 2.1) exceed the default highly protective performance target set by the WHO[19] for bacterial reduction (i.e., differences in excess of 4 LRV). A difference in laboratory versus field performance of 4 LRV is estimated by the WHO[19] itself for bacterial indicators with respect to size exclusion approaches (i.e., ceramic filtration, bacterial reduction, Table 2.1). Such data implies that some POUWT methods found to be highly protective based on laboratory data have the potential to confer zero LRVs (and therefore limited to no protection) in the field. Other differences in reported laboratory versus field data[21,24,82,84] are equal to or greater than that needed to “graduate” from protective to highly protective (i.e., $\geq$ 2 LRV) under the WHO performance targets[19], implying that some techniques found to be highly protective based on laboratory data could meet lower protective or interim status based on field data.

Table 2.1 Reported discrepancies between laboratory efficacy and microbiological field performance, with comparison to aggregate estimates published by the WHO[19]

Reference	Reported mean laboratory efficacy (LRV)[a]	Reported mean microbiological field performance (LRV)[a]	Discrepancy between reported microbiological field performance and laboratory efficacy (LRV)
Ceramic Filter			
Brown et al. (2008 & 2010)[87,104]	2.2	1.4	0.8
Murray et al. (2017)[25]	9.0[b]	1.0	8.0[b]
Farrow et al. (2018)[84]	2.3	1.3[b]	1.0
Guerrero-Latorre et al. (2019)[96]	5.4	1.3	4.1
Kallman et al. (2011)[82]	3.5	1.1	2.4
WHO (2011)[19] Aggregate estimate for porous ceramic filtration	6 Bacteria 4 Virus 6 Protozoa including *cryptosporidium*	2 Bacteria 1 Virus 4 Protozoa including *cryptosporidium*	4 Bacteria 3 Virus 2 Protozoa including *cryptosporidium*
Bio Sand Filter			
Stauber et al. (2006)[28]	1.3	1.2	0.1
Murray et al. (2020)[24]	3.5	0.7	2.8
WHO (2011)[19] Aggregate estimate for household slow sand filtration	3 Bacteria 2 Virus 4 Protozoa including *cryptosporidium*	1 Bacteria 0.5 Virus 2 Protozoa including *cryptosporidium*	2 Bacteria 1.5 Virus 2 Protozoa including *cryptosporidium*
Chlorine Disinfection			
McLaughlin et al. (2009)[81]	5.2	1	4.2
Murray et al. (2020)[24]	6.0[b]	0.6	5.4[b]
WHO (2011)[19] Aggregate estimate for free chlorine disinfection	6 Bacteria 6 Virus 5 Protozoa, non-*cryptosporidium* 1 *Cryptosporidium*	3 Bacteria 3 Virus 3 Protozoa, non-*cryptosporidium* 0 *Cryptosporidium*	3 Bacteria 3 Virus 2 Protozoa, non-*cryptosporidium* 1 *Cryptosporidium*
Electrolytic Silver Disinfection			
Hill et al. (2022)[103]	5.6	0.2[b]	5.4

[a] *All study organisms* E. coli *or faecal coliforms unless otherwise stated*

[b] *Censored data reported*

Laboratory-generated performance data (LRVs) of POUWT strategies are used as input data for QMRA studies[30,105–107], based on which recommendations can be made by public health organizations or local governments regarding method selection or guidance for treatment. If POUWT performance has been overestimated using laboratory-based data, and end-users are seeing decreased performance, then such public health recommendations could be inaccurate or problematic. For example, recommendations may end up favouring a method that has a higher laboratory efficacy but lower field usability and performance[80], which could compromise the health protection offered to the end user.

HWTS practitioners are now re-framing the paradigm of POUWT approaches from a "silver bullet" technology, which was based on high LRVs generated via laboratory studies, to one that includes research on sustainability and POUWT approaches that take context into account and reduce the need for behaviour change[108]. Such a shift follows Gartner's Hype Cycle, from the initial "technology trigger"; the "peak of inflated expectations" (i.e., the silver bullet); "trough of disillusionment" (i.e., observed decline in adherence over time and non-significant health outcomes from randomized controlled field trials); to the "slope of enlightenment" and presently to the "plateau of productivity", including field evaluations of POUWT devices to ascertain the true LRVs and therefore potential protection conferred to the end-user[108].

2.5 What can be done to address the laboratory versus field data discrepancy?

During laboratory efficacy testing, spiked water having anywhere from 10^5-10^9 organisms per 100 mL has been used to challenge test POUWT methods and thus calculate LRVs on the order of 5 to 9 LRV[19]. As noted above, field performance studies are limited by the use of lower environmental levels of microorganisms (i.e., lack of a high spike). This can lead to censored LRVs characterized by non-detected effluent microorganisms, which was observed in some studies (Table 2.1). Therefore, challenge water with a higher organism spike has been suggested for field evaluations[24,36,109,110].

Such spike organism(s) should be safe for human consumption (i.e., "food-safe") to be used outside a laboratory setting and to test POUWT techniques under actual use, to satisfy ethical and safety requirements for human study participants. In addition, spike organisms should be easily transportable and culturable using feasible techniques that can be

deployed outside the laboratory setting. An appropriate bacterial surrogate (a probiotic health supplement containing non-pathogenic *E. coli*) has been identified and was subject to preliminary validation through previous work[36] via an established surrogate selection framework[111]. Baker's yeast (*Saccharomyces cerevisiae*) has been identified as a possible non-pathogenic surrogate for protozoans[37,38], has been applied as a challenge organism to evaluate *in situ* microbiological performance in non-potable water applications[39,40] and has been recommended for further use for other *in-situ* evaluations[41]. A suitable viral surrogate has not yet been proposed in the published literature; this is a research gap that would be valuable to address, completing the "suite" of food-safe microbiological surrogates.

Using probiotic *E. coli* and baker's yeast as food-safe surrogates for bacteria and protozoa, respectively, we propose the concept of "field challenge testing". Under this concept, POUWT techniques would be challenge tested *in situ* using food-safe surrogates as a compliment to data obtained in the laboratory.

2.6 Useful applications of the field challenge test method

Given the great global need for effective HWTS, there is a corresponding need for effective POUWT evaluation protocols to assess microbe reduction of these technologies under conditions that are representative of real life situations, including user conditions and water quality[112]. The field challenge test method aims to address this need.

One potential application for field challenge testing would be to ascertain the (non-censored) performance of POUWT strategies under real use conditions. Field challenge studies would comprise sending specifically-trained enumerators to visit households and conduct field challenge studies using the POUWT method on premise, in a similar fashion to existing water quality data collection techniques currently employed by the WHO/UNICEF Joint Monitoring Programme (JMP)[113,114]. POUWT users could be engaged as study participants to use their own POUWT method to treat a volume (e.g., 1 L) of spiked water, containing probiotic supplement and/or baker's yeast as outlined above. Field challenge testing could be combined with other established survey methods, such as water quality testing at points of collection and consumption[114], a participant questionnaire[115] and/or a HWTS sanitary inspection[116]. Following testing, enumerators would ensure that microbes are flushed and/or cleaned from the POUWT device with

either 70% ethanol, or soap and water as appropriate; participants would not drink the spiked test water.

Enumerators would be trained for the express purpose of conducting microbiological challenge tests, proficient in water quality testing methods, including aseptic technique. They would use established field water quality testing methods to process influent and effluent water samples resulting from the field challenge test, such as field membrane filtration to enumerate *E. coli*[113,114], and SimPlate method for Yeast and Mold Color Indicator (Y&M-CI) method is used for the detection and quantification of yeast[117], which has been validated for use against conventional agar plating methods[118,119] and is appropriate for low-resource field contexts due to the pre-packaged sterile materials it uses, as well as the lack of requirement for reagent refrigeration[117].

Field challenge testing would garner more field-relevant data (i.e., under real-use conditions, where it matters most), thereby reflecting the influence of the contexts in which they are used, as opposed to idealized laboratory conditions. Risky user behaviours and/or environmental factors could be identified via observations or questionnaires, such as cross-contamination during water treatment or low-quality influent water, as well as quantifying impacts on device performance. Results could be compiled to monitor and classify health risk following approaches similar to that used by the WHO[120] to integrate sanitary inspection and water quality data. Identification of the riskiest factors could help to guide feedback for the design of POUWT, and/or instructional campaigns for best practices regarding POUWT methods.

It is now accepted that compliance is essential in QMRA modelling to effectively estimate health gains, which are then used to make public health recommendations[31,32,34,121]. We propose that site-specific microbiological field performance data, gathered via field challenge testing by use of food-safe surrogates (i.e., probiotic bacteria[122] and/or baker's yeast[37–41]) could and should be incorporated into QMRA models the same way, although a research gap exists regarding food-safe viral surrogates. QMRA models are currently essential in understanding risk and facilitating important public health decisions; adding site-specific challenge test data in combination with compliance data would be highly instructive[123]. Field challenge testing would also be useful in application to technologies for which compromised performance is not solely driven by user error; namely, ceramic water filters (CWFs)[24,25,100,101]. CWFs are typically manufactured in decentralized facilities, where lack of access to a centralized laboratory for microbiological quality control

causes a triple burden of logistical complexity, cost and time delays[57,58,124,125] while excluding local stakeholders from long-term gains in skills and knowledge[126]. Ideally, CWFs would be tested on-site or locally[126] allowing CWF manufacturers to implement low-cost microbiological quality control, for which there have been recent calls[125]. Similar microbiological methods would be used as described above, except that challenge testing would take place at established CWF manufacturing facilities, rather than by household survey. This would support filter manufacturers to produce, and therefore consumers to purchase, consistently high-quality technologies, while keeping knowledge and quality control practices local to the community[126].

2.6.1 Limitations of the field challenge test method

All studies are subject to limitations, and it is important to report limitations for study transparency. As noted above, spike organisms would be rehydrated in local source water using commercially-available probiotic supplements or baker's yeast, which poses several limitations. The probiotic and the yeast are both dry powder, and therefore their addition to water will increase turbidity, even though small amounts of powder are used, potentially causing interactions with chlorine (i.e., chlorine demand), UV disinfection (i.e., shielding), or filtration (i.e., clogging)[19]. Traditional (laboratory-based) challenge testing entails pre-culturing spike organism(s) in a nutrient medium (i.e., non-selective agar or broth) to bring the microorganisms to stationary phase and purifying the mixture (i.e., by centrifugation or by agar washing) before spiking[18,19]. Aside from minimizing interactions with various treatment mechanisms as described, this process ensures that organisms are at their most robust and resistant to treatment (in particular disinfection), thus providing a conservative LRV estimate[18,19]. Such pre-culturing is not ethically possible with field challenge testing, where study participants would use their own POUWT devices for testing. This is due to the potential to inadvertently culture a pathogenic microorganism during the pre-culture phase, either via cross-contamination or random mutation of cultured microbes during growth – even if the seed microbe is a food-safe and aseptic technique is employed.

Although validated and widely used, limitations in field-based culture include the somewhat increased potential for sample contamination during processing, although this can be mitigated by quality control (QC) techniques such as regular (i.e., daily or every 10 tests) negative control (i.e., processing an additional "blank" test of locally-purchased

bottled water, assumed to be free of detectable levels of contamination), typically used by MICS surveys[113,114], can be applied to field challenge testing. In addition to blank tests, in the MICS data collection programmes, data reliability is aided by intensively training enumerators prior to deployment, using standardized and pre-sterilized materials for microbe quantification, and continually monitoring quality of enumerators, typically by a supervisor who is an experienced laboratory technician[114]. Additionally, field enumeration techniques typically require additional time to process samples compared to laboratory techniques, meaning that triplicate samples are difficult and time-consuming to process. The limited capacity for triplicate analysis could be mitigated by employing additional enumerators to conduct field challenge testing or bringing samples back to a laboratory for analysis, depending on the study location.

Beyond any culture technique-based study limitations, the use of field challenge data as inputs to QMRA modelling is also subject to limitations. The use of non-pathogenic microbes as a process indicators for reference pathogens is established for *in situ* studies or in cases where using a pathogenic spike organism is infeasible[127]. In cases where non-pathogenic surrogates are used to measure LRVs (i.e., for *in situ* studies), the non-pathogenic surrogate functions more as a general (process) indicator, demonstrating the overall process efficacy[127]. The choice of indicator-pathogen relationship is an assumption that impacts reference pathogen LRV estimates, which can have a relatively high impact on QMRA models[128], the extent of which should be assessed via sensitivity analysis.

All studies are subject to limitation; however, even if the techniques are imperfect, that does not mean they are not useful and informative. Field-generated data can fill critical data gaps and augment laboratory-based findings[129], allowing a more comprehensive characterization of the state of public health conditions in low-resource contexts. We therefore believe that our proposed method is a useful way to fill the research gap as outlined in the present Perspective.

2.7 Outlook and Summary

In this Perspective, we highlight a discrepancy between the laboratory versus field performance of POUWT methods and discuss the resulting overestimation of potential health protection conferred upon the end user if laboratory-based estimates are solely used. We propose field challenge testing as a strategy to address this research gap, using food-safe bacterial and protozoan surrogates. Such a method would generate information that

is more representative of POUWT performance experienced by the end user, which can be influenced by factors including environmental water quality, correct and sustained use of POUWT, access to maintenance and repair, and correct cleaning, water retrieval and maintenance activities (Figure 2.1). When such factors are represented in the data, then POUWT techniques can be viewed within the larger "source to sip" framework of HWTS, which encompasses the water source (in terms of protection, accessibility and sustainability), POUWT approaches and safe water storage and consumption[15]. By doing so, sustainable and scalable interventions can be made to reduce exposure to faecal contamination via drinking water and realize health gains, of which there is an urgent need[130].

2.8 Author contributions

Conceptualization, C.Z. and C.D.; literature review, C.Z.; data compilation, C.Z.; writing—original draft preparation, C.Z. and C. D.; writing—review and editing, C.D.; supervision, C.D.; funding acquisition, C.D.

2.9 Supporting information

Table S2.2: Additional information on studies comparing laboratory efficacy with field performance of ceramic filters. Study organism for field evaluations E. coli *or faecal coliforms unless otherwise noted.*

Study	**Device Details**	**Laboratory information**						**Field information**						**Discrepancy between reported microbiological field performance and laboratory efficacy[c], log_{10}**
		No. Filters Tested	**Water Information**	**Study Organism**	**Spike Concentration (CFU, MPN or PFU/100 mL)**	**Reported laboratory efficacy (log_{10})**	**Reported non-microbiological performance**	**Location**	**No. House-holds**	**Comparison Points**	**Water Information**	**Reported field performance (log_{10})**	**Reported non-microbiological performance**	
Brown et al. (2008 & 2010)	Ceramic "pot" filter, manufactured by Resource Development International (RDI)	4 with $AgNO_3$ treatment	Rainwater and surface water	*E. coli* CN13 (ATCC 700609)	10^6 –10^9	Rainwater 2.2 (95% CI 2.0-2.4) Surface water 2.3 (95% CI 2.1-2.5)	Flow rate, turbidity not reported	Prek Thmey village, Kandal Province, Cambodia	50	Stored household water vs. spigot	Surface water and/or rainwater	1.4	89% turbidity reduction 1.5-3 L/hour flow rate	0.85 (compared to average LRV between rainwater and surface water in the laboratory)
				Bacteriophage MS2 (ATCC 15597-B1)	10^7–10^{10}	Rainwater 1.3 (95% CI 0.83-1.8) Surface water 1.5 (95% CI 1.1-1.9)						MS2 not studied in the field		
Murray et al. (2017)	Sawyer PointONE™ hollow fiber ceramic filter with bucket assembly	1	Sterile deionized water	*E. coli* (strain not specified)	10^7 10^8 10^9	7.0[a,b] 8.0[a,b] 9.0[a,b]	719 mL/min flow rate expected (Sawyer Products, n.d.)	Honduras	50		Surface water	0.97[b]	89% turbidity reduction 77.2 mL/min flow rate (95% CI 62-96 mL/min)	8.0 (as compared to 10^9 laboratory spike)
Farrow et al. (2018)	Ceramic "pot" filter (manufacturer not reported)	2	Sterile deionized water	*E. coli* (ATCC 700891)	10^4-10^5	2.3 (minimum 1.6, maximum 3.0[a])	Flow rate, turbidity not reported	Longhai City, China	8		Well water or spring water	1.3 (minimum 0.6, maximum 3.0[a])	Not reported	1
Guerrero-Latorre et al. (2019)	Black ceramic "pot" filter, manufactured by the Horeb factory in Ecuador	3	Dechlorinated tap water	E. coli (CECT 25922)	$1.5 \cdot 10^8$	5.4 (± 0.4 SE)	98% reduction in turbidity	Santa Marianita, Ecuador	19		Tap water	1.3 (± 0.1 SE)	Not reported	4.1
Kallman et al. (2011)	Ceramic "pot" filter impregnated with silver (manufactured in San Mateo, Ixtatan, Guatamala)	Not reported	Phosphate-buffered saline solution	*E. coli* (wild type)	$7 \cdot 10^9$	3.5[b] for 9% sawdust content by mass	Flow rate, turbidity not reported	San Mateo Ixtatan, Guatemala	62	Top bucket vs. spigot	Tap water	1.1 (± 0.04 SD)	90% turbidity reduction	2.4
WHO (2011)	Porous ceramic and carbon block filtration			Bacteria		6				Not reported		2		4
				Virus		4				Tap vs. point of use		1		3
				Protozoa		6				Tap vs. spigot		4		2

[a] *Censored data reported;* [b] *Statistical information not reported;* [c] *Calculated by subtracting mean reported LRVs for lab vs field.*

Table S2.3: Additional information on studies comparing laboratory efficacy with field performance of biosand filters. Study organism for field evaluations E. coli *or faecal coliforms unless otherwise noted.*

Study	**Device Details**	**Laboratory information**							**Field information**						**Discrepancy between reported microbiological field performance and laboratory efficacy[c], log_{10}**
		No. Filters Tested	**Water Information**	**Operation Details**	**Study Organism**	**Spike Concentration (CFU or MPN/100 mL)**	**Reported laboratory efficacy (log_{10})**	**Reported non-microbiological performance**	**Location**	**No. Households**	**Comparison Points**	**Water Information**	**Reported microbiological performance (log_{10})**	**Reported non-microbiological performance**	
Stauber et al. (2006)	Laboratory: Purchased from Danvor Water Treatment, AB, Canada Field: Locally-made filters studied	2	Lake water	Influent flow rate 40 L/day, operated for 17 days or 43 days (scenario 1 or 2, respectively)	*E. coli* B (ATCC 11303)	10^7 scenario 1 10^5 scenario 2	Average 1.3 Minimum 0.4[b]	Flow rate decline by over 75% reported during laboratory study Turbidity not reported	Bonao, Dominican Republic	55	Household water vs. filter spigot	Not reported	Average 1.2 Minimum 0 Maximum 2.5	Average turbidity reduction 84% Flow rates not reported	0.1
Muray et al. (2020)	Lightweight plastic bucket construction	4	Not reported	Filters operated over a 250-day period	*E. coli* (ATCC 11775)	106	3.5 (range 3.6-3.3)[b]	Average turbidity reduction of 97%[b]	San Juan del Sur, Nicaragua	88	Source water vs. cup	Protected or unprotected well, piped kiosk or surface water	0.7	Not reported	2.8
WHO (2011)	Household-levelled intermittently operated slow sand filtration				Bacteria		3						1		2
					Virus		2						0.5		1.5
					Protozoa		4						2		2

[a] *Censored data reported;* [b] *Statistical information not reported;* [c] *Calculated by subtracting mean reported LRVs for lab vs field.*

Table S2.4: Additional information on studies comparing laboratory efficacy with field performance of chlorine disinfection. Study organism for field evaluations E. coli *or faecal coliforms unless otherwise noted.*

Study	**Method Details**	**Laboratory information**									**Field information**					**Discrepancy between reported microbiological field performance and laboratory efficacy[c], $\log_{10}$**
		No. Runs	**Water Information**	**pH**	**Temperature (°C)**	**Contact time**	**Initial Chlorine Dose (mg/L)**	**Study Organism**	**Spike Concentration (CFU or MPN/100 mL)**	**Reported laboratory efficacy ($\log_{10}$)**	**Location**	**No. Households**	**Comparison Points**	**Water Information**	**Reported microbiological performance ($\log_{10}$)**	
McLaughlin et al. (2009)	Liquid hypochlorite	4, analysed in duplicate	Autoclaved lake water	Not reported	26.5°C and 4°C	24 hours	2.9	*E. coli* (field-isolated strain)	108	Median value 5.2	Borbon region of Ecuador	9; total of 46 paired samples taken	Water source vs. point of use	Local stream	Median value 1.0	4.2
Murray et al. (2020)	Electrochlorinator	Evaluated under WHO Scheme conditions (WHO, 2016)					2.5	Bacteria		> 6.0[a]	Leogane, Haiti	60 paried samples	Water source vs. cup	Local spring water (unprotected) or piped kiosk	0.6[b]	5.4[a]
WHO (2011)	Free chlorine disinfection							Bacteria		6					3	3
								Virus		6					3	3
								Protozoa, non-*cryptosporidium*		5					3	2
								Cryptosporidium		1					0	1

[a] *Censored data reported;* [b] *Statistical information not reported;* [c] *Calculated by subtracting mean reported LRVs for lab vs field.*

Table S2.5: Additional information on studies comparing laboratory efficacy with field performance of electrolysis. Study organism for field evaluations E. coli *or faecal coliforms unless otherwise noted.*

Study	**Method Details**	**Laboratory information**									**Field information**					**Discrepancy between reported microbiological field performance and laboratory efficacy[c], log_{10}**
		No. Runs	**Water Information**	**pH**	**Alkalinity (meq/L)**	**Contact time**	**Initial Silver Dose (µg/L)**	**Study Organism**	**Spike Concentration (CFU or MPN/100 mL)**	**Reported laboratory efficacy (log_{10})**	**Location**	**No. Households**	**Comparison Points**	**Water Information**	**Reported microbiological performance (log_{10} *E. coli*)**	
Hill et al. (2022)	Silver electrolysis	2	Synthetic groundwater	7.4-7.8	57-64	8 hours	approx. 50	*E. coli* C300	Not specified	5.6	Dzimauli community, Limpopo, South Africa	20	Source water vs. spigot	None	Average 0.2 over 4 week study period[a]	5.4

[a] *Censored data reported;* [b] *Statistical information not reported;* [c] *Calculated by subtracting mean reported LRVs for lab vs field.*

Table S2.6: Additional information on studies comparing laboratory efficacy with field performance of electrolysis. Study organism for field evaluations E. coli *or faecal coliforms unless otherwise noted.*

Study	**Method Details**	**Laboratory information**						**Field information**						**Discrepancy between reported microbiological field performance and laboratory efficacy[c], log_{10}**
		No. Filters Tested	**Water Information**	**Study Organism**	**Spike Concentration (CFU or MPN/100 mL)**	**Reported laboratory efficacy (log_{10})**	**Reported non-microbiological performance**	**Location**	**No. Households**	**Comparison Points**	**Water Information**	**Reported microbiological performance (log_{10})**	**Reported non-microbiological performance**	
Murray et al. (2020)	Combined ceramic disk filter with bromine disinfection (CBS)	Laboratory testing done by others; methods not reported	Not reported	Bacteria (strain not reported)	Not reported	> 7.0[a]	Not reported	Kenya Haiti	50 total	Water source vs. cup	Local spring water (unprotected) or piped kiosk	0.5 in Kenya 1.4 in Haiti	Not reported	6.5[a] in Kenya 5.6a in Haiti

[a] *Censored data reported;* [b] *Statistical information not reported;* [c] *Calculated by subtracting mean reported LRVs for lab vs field.*

Chapter 3: Validation of EcN as a suitable surrogate for EcK12 in field evaluation of disinfection-based point-of-use water treatment

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3.1 Abstract

Decentralized approaches can be used to make drinking water safe using the "source to sip" framework that includes point-of-use water treatment (POUWT) methods. Of the myriad methods that are available for POUWT, disinfection methods have the lowest cost and are relatively easy to perform, therefore having a low barrier to entry and potential for high user acceptability.

The microbiological performance of disinfection and other POUWT methods are assessed in the laboratory under controlled conditions, potentially leading to an optimistic estimate of treatment performance in comparison to performance seen when POUWT technologies are employed by the end user. Therefore, the concept of "field challenge testing" has been proposed and piloted, using probiotic *E. coli* Nissle (EcN) as a food-safe bacterial challenge organism. However, EcN has not yet been assessed for use with free chlorine (FC) or UV

disinfection, and therefore the objective of this study was to validate EcN for use in evaluating disinfection-based POUWT methods *in situ*.

We carried out laboratory-based disinfection dose-response experiments of EcN and standard laboratory strain *E. coli* K-12 (EcK12) using either UV collimated beam or FC exposure. With respect to free chlorine disinfection, EcN and EcK12 responded similarly under all conditions studied, although when EcN was dissolved directly from the powder capsule into test water prior to testing, the low initial *E. coli* concentration limited comparisons due to censored observations (i.e., right-censored LRVs). We observed a sharp drop in microbe concentrations when exposed to the lowest possible free chlorine dose, with declining LRVs observed with increasing dose. When exposed to UV disinfection, there were some statistically significant differences in inactivation rate constants and dose required for $3\log_{10}$ reduction between some conditions studied. However, the statistical differences were not substantive when compared to between-study differences in dose-response behaviour for the same *E. coli* strains within the published literature. We therefore conclude that EcN is a practical surrogate for EcK12 in application to field challenge testing of POUWT devices via free chlorine or UV disinfection.

3.2 Keywords

Chlorine disinfection; UV disinfection; *Escherichia coli*; point of use water treatment.

3.3 Introduction

Unsafe drinking water, inadequate availability of water for hygiene, and lack of access to sanitation together contribute to about 88% of deaths from diarrheal diseases, with the burden of disease falling disproportionately to children under five years old[5]. Waterborne diarrheal illnesses are preventable via adequate sanitation, hygiene and safe drinking water[131] and are thus the focus of one of the Sustainable Development Goals (SDG 6: "Ensure availability and sustainable management of water and sanitation for all")[2]. Despite this, more than 2 billion people globally lack access to safe and effectively treated water[13].

Decentralized approaches can be used to make drinking water safe, including point-of-use (PoU) water treatment and safe water storage[4]. These approaches can be used in contexts where centralized treatment is unavailable, difficult, expensive or infeasible[4]; piped water supply is intermittent and/or of poor quality[132] or in a humanitarian emergency[64]. Also

referred to as household water treatment (HWT), PoU water treatment methods effectively prevent the transmission of waterborne illness[65] as part of the “source to sip” framework that includes an improved water source, effective conveyance (e.g., piped network or a clean fetching container), safe storage, household access and a clean drinking glass[15].

The main categories of PoU water treatment are disinfection, including chlorination or ultraviolet (UV) disinfection; filtration, including biosand and ceramic filtration; flocculation (e.g., the P&G Purifier of Water), or some combination thereof[52]. Of these categories, disinfection methods have the lowest cost, at approximately USD $0.63 and $0.66 annually per person for UV and chlorination, respectively[133]. Aside from cost, both methods have a low barrier to entry, with very few materials required for water treatment, and are relatively easy to perform, therefore having a high potential for user acceptability[52].

The microbial reduction provided by PoU technologies (disinfection or otherwise) is typically assessed by a method called “challenge testing”, which consists of spiking water with virus, bacteria or protozoa, or their respective surrogates, and using that water to treat the PoU technology of interest[18,19]. Microbe reduction (i.e., $\log_{10}$ reduction values, LRVs) is contextualized via WHO performance categories (i.e., highly protective, protective or interim)[19]. Estimations can also be made regarding the health impacts of a given PoU device via the process of quantitative microbial risk assessment (QMRA)[30,105–107,134].

Challenge testing is carried out in the laboratory under controlled conditions, potentially leading to an optimistic estimate of treatment performance in comparison to performance seen when PoU technologies are employed by the end user[20]. This could be due to reasons such as poor or variable water quality for both chlorine[24,81] and UV[135], improper dosage of chlorine[81], variability of weather conditions and sunlight exposure with SODIS[67] and potential water recontamination, for UV methods[67] or for chlorine in cases where chlorine demand is high and/or storage time is long[62].

A concept called “field challenge testing” has been proposed[20] and implemented (Chapter 6) in order to assess microbe reduction of PoU treatment methods at the actual point of use, to potentially encapsulate *in situ* treatment performance and generate data that are more reflective of field situations, for which there have been recent calls[112]. Under “field challenge testing”, challenge water can be spiked with food-safe microbes and used for

treatment outside a laboratory setting or during interactions with a consenting study participant (who would not drink the spiked water). An appropriate bacterial surrogate (a probiotic health supplement containing non-pathogenic *E. coli*) has been identified and was subject to preliminary validation through previous work[36] via an established surrogate selection framework[111]. The proposed food-safe probiotic bacterial surrogate (strain name Nissle 1917, or EcN, brand name Mutaflor®) has not yet been assessed for use with free chlorine (FC) or UV disinfection, and this was the aim of the present study.

The overall objective of this work was to validate the field-appropriate probiotic surrogate EcN for use in evaluating disinfection-based POUWT methods *in situ*. The specific objectives of this work were as follows:

1. Determine the disinfection efficacy of FC and UV on probiotic EcN.
2. Compare the disinfection efficacy of FC and UV on EcN to that of *E. coli* K-12 (EcK12), a standard laboratory strain.
3. Assess the change (if any) in disinfection efficacy of EcN arising a simplified preparation method; namely, dissolving the probiotic powder capsule into water directly prior to disinfection, compared to incubation of the bacteria to stationary phase. Pre-culturing microbes prior to spiking is not possible during field challenge testing due to field constraints such as a potential lack of consistent electricity supply for incubation, and/or ethical constraints such as the potential to inadvertently culture a pathogenic microorganism on nonselective media[20].

3.4 Methods

3.4.1 Overview

To address the three objectives described above, FC and UV disinfection dose-response experiments were conducted under three conditions: 1) EcN incubated to stationary phase; 2) EcN dissolved from powder capsule directly prior to chlorine exposure; and 3) EcK12 incubated to stationary phase. For chlorine dose-response experiments, microbes were exposed to FC at three target concentrations: 0.0 (control), 0.1 and 0.5 mg/L (Figure 3.1). Runs were each carried out three times, apart from control runs (0.0 mg/L FC), which were carried out singly (see Section 3.4.4 for a full description of one typical run). Regarding UV disinfection, under each condition seven dose-response experiments were conducted in triplicate, at UV exposures of 0 (control), 20, 30, 40, 50, 60 and 70 mJ/cm^2,

for a total of 21 experimental runs per condition, randomized in order (Figure 3.2; see Section 3.4.5 for a full description of one typical run). Throughout this paper, we use the widely-used term "dose" (with units of mJ/cm^2) to refer to UV fluence, although "fluence" is the proper term[136]. This is for the sake of simplification, as "dose" is the proper term used in chemical disinfection studies (with units of $mg \cdot min/L$).

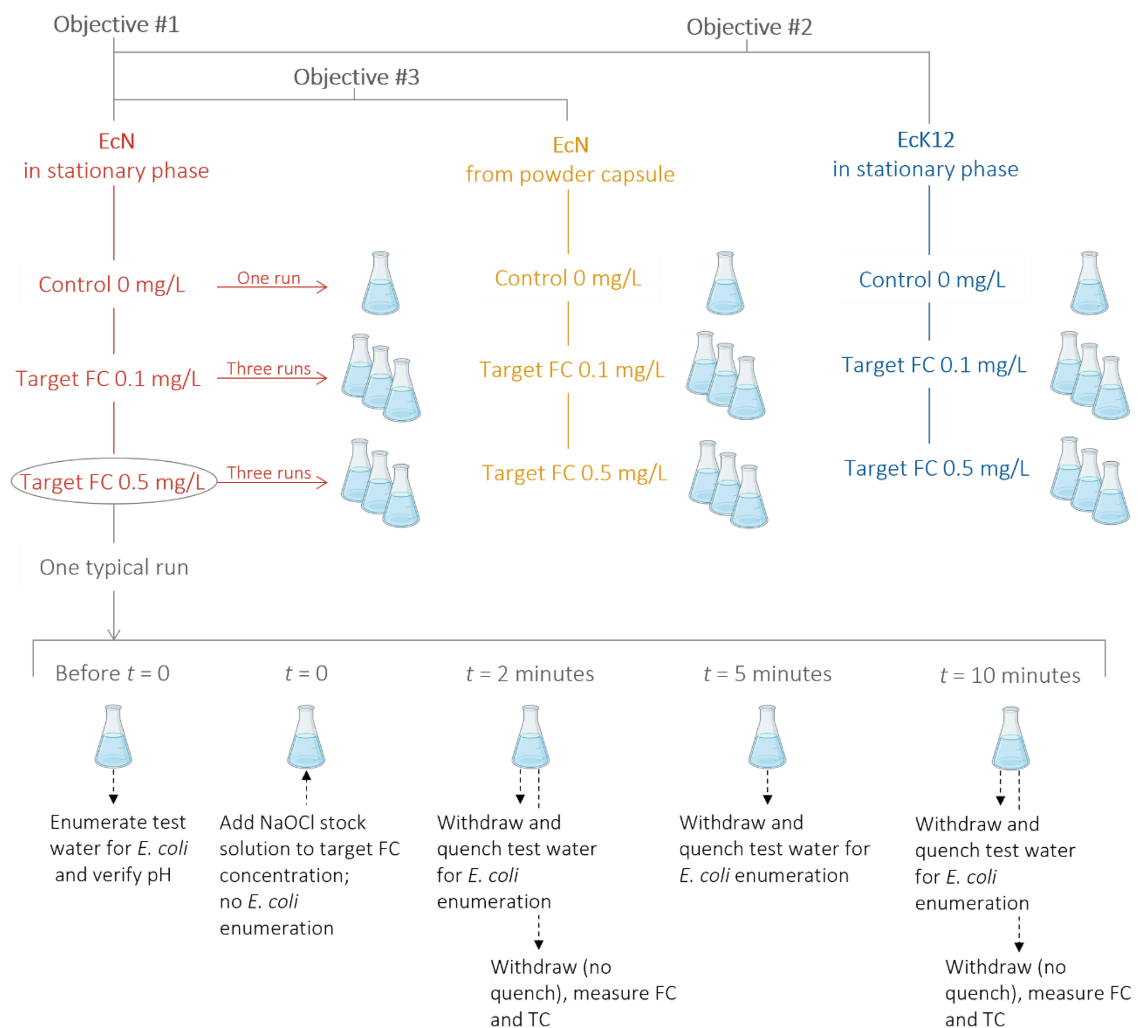

Figure 3.1: Overview of free chlorine (FC) dose-response disinfection experiments. One typical run is circled in grey and is applicable for all runs, including control runs (no NaOCl added in this case). TC stands for total chlorine.

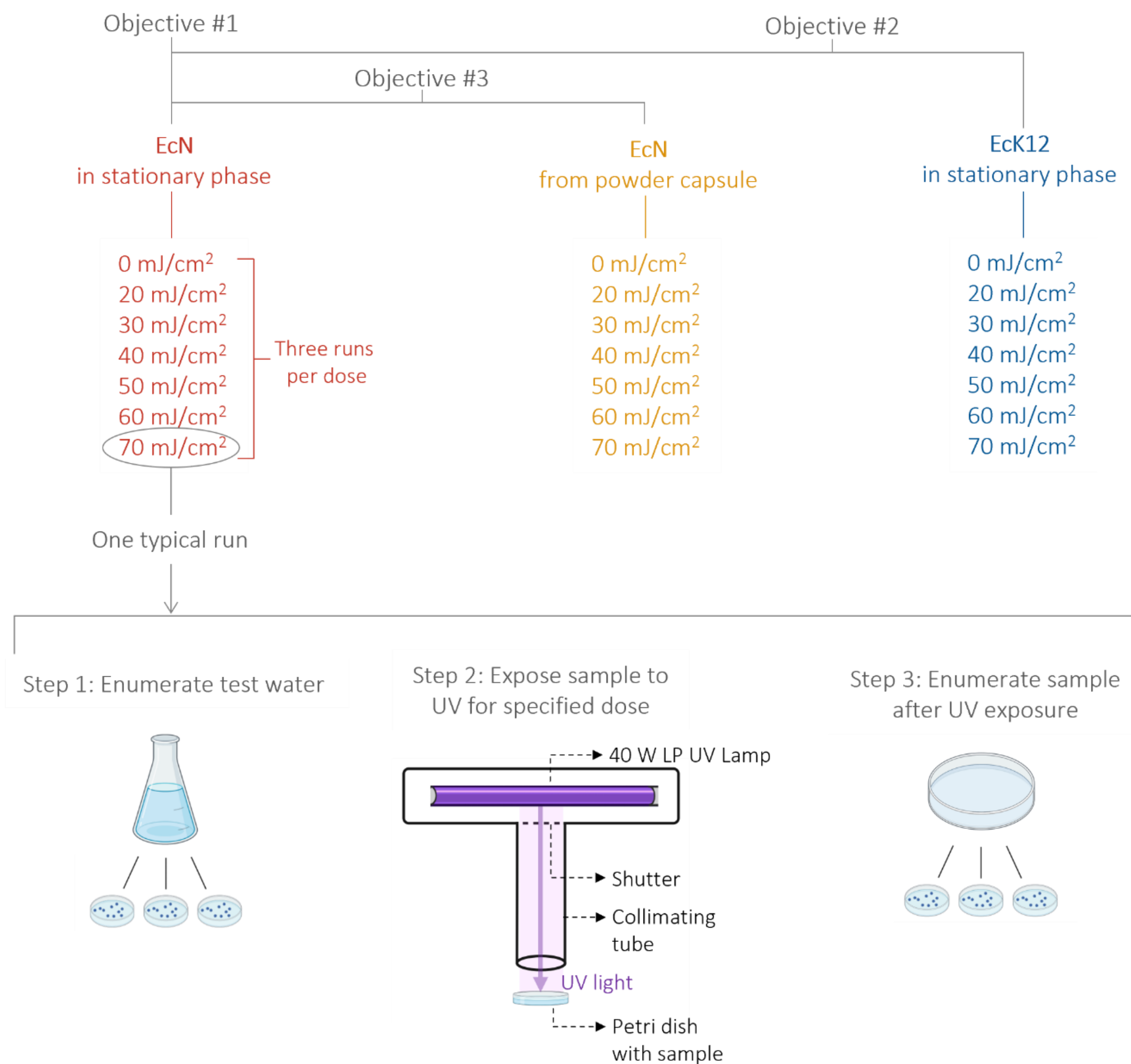

Figure 3.2: Overview of UV dose-response disinfection experiments. One typical run is circled in grey and is applicable for all runs, including control runs (no UV exposure in this case). Collimated beam experiments were performed on a standing water sample; samples were enumerated before and after UV exposure using membrane filtration culture methods (see Section 3.4.2).

3.4.2 Bacteriological methods

Two strains were used in this study: probiotic *E. coli* (strain name Nissle 1917, or EcN, brand name Mutaflor®, Pharma-Zentrale GmbH, Herdecke, Germany), and a standard laboratory strain, *E. coli* K-12 (EcK12, ATCC 700926).

EcN preparation methods

EcN dry powder capsules were kept refrigerated once received until use, and tests were conducted before stated product expiry dates. The day prior to testing, one capsule was externally sterilized by wiping the outside with 70% ethanol (Commercial Alcohols, Brampton, ON, Canada) and allowed to dry before aseptically opening and carefully depositing the powder contents into approximately 100 mL of sterile buffered, quarter-strength isotonic Ringers solution (Oxoid Ltd., England, United Kingdom). The mixture was stirred on a magnetic stirrer on the lowest speed setting (500 rpm, Ika Topolino S1, IKA, Staufen, Germany) for approximately 5 minutes at room temperature to dissolve the powder. Once dissolved, a loopful of mixture was inoculated onto a nutrient agar slant (Tryptic Soy Agar, TSA, Sigma Aldrich, St. Louis, Missouri, USA) and incubated (Forced Air Microbiological Incubator 6.3CF, VWR, Radnor, Pennsylvania, USA) at 35°C overnight (16-18 hours) to reach stationary phase[36]. Cells were harvested by washing the agar surface twice with approximately 2 mL of sterile Ringers (i.e., total of approximately 4 mL), then diluted to about 100 mL using sterile Ringers[137]. This first dilution was stirred on a magnetic stirrer at 500 rpm for approximately 5 minutes at room temperature. From this first dilution, 5 mL was aseptically transferred to a freshly prepared 2 L Erlenmeyer flask (for chlorine dose-response experiments) or a 500 mL media bottle (for UV dose-response experiments) of sterile test water (Butterfield's buffer at pH 7.0, preparation described below). Test waters were again stirred for 5 minutes at 500 rpm before disinfection exposure (described below). Using this method, initial EcN concentrations were between 10^7 and 10^8 CFU/100 mL.

For runs where no incubation step took place, EcN was dissolved directly prior to chlorine exposure by aseptically drawing 100 mL of sterile test water from the 2 L or 500 mL test flask then aseptically opening, depositing, and stirring one capsule into the test water as described in the previous paragraph. Once dissolved, the mixture was deposited back into the 2 L test flask (for FC dose-response experiments) or 500 mL media bottle (for UV dose-response experiments) with the bulk of the test water and stirred for 5 minutes at 500 rpm before disinfection exposure (described below). Initial EcN concentrations were approximately 10^3 and 10^7 CFU/100 mL for FC and UV dose-response experiments, respectively.

EcK12 preparation methods

EcK12 were stored at -80°C in Lysogeny broth (LB, Difco Laboratories, Franklin Lakes, NJ, USA) containing 50% (vol./vol.) glycerol and sub-cultured onto TSA plates (stored at 4°C) at approximately 3-month intervals. The day prior to testing, a loopful of EcK12 culture was inoculated from the TSA plate to a TSA slant, then incubated at 35°C overnight (16-18 hours) to reach stationary phase. After incubation, cells were harvested and transferred to a 2 L or 500 mL flask (for FC and UV tests, respectively) of sterile test water as described above for EcN.

Enumeration

All samples were enumerated in triplicate using membrane filtration with m-ColiBlue broth (Hach Company, Colorado, USA) and incubated for 24 hours at 35°C[138]. After 24 hours, presumptive *E. coli* colonies were counted by visual inspection. If necessary (i.e., for test water before disinfection, control samples, and for UV dose-response samples post-exposure), serial 10-fold dilutions were carried out prior to plating using sterile Ringers solution. For FC dose-response runs, 100 mL of post-disinfection water was plated. The lower limit of detection for plated samples was 1 CFU per volume of water plated.

3.4.3 Test water

FC and UV dose-response experiments were conducted with Butterfield's phosphate buffer (BPB, adjusted to pH 7.0) as the medium[137]. First, a hydrogen-ion buffer was made by combining autoclaved 290 mL and 500 mL of 0.1M solutions of NaOH and KH_2PO_4, respectively (both Fisher Chemical, Waltham, Massachusetts, United States), and diluting the mixture to make 1 L using autoclaved type 1 ultrapure water (MilliQ Super-Q water purification system, Millipore Sigma, Burlington, MA, USA). BPB was then prepared by diluting 100 mL of hydrogen-ion buffer to 2 L, or 25 mL to 500 mL as appropriate, with autoclaved MilliQ water and adjusting the pH to 7.0 using autoclaved 0.1 M H_2SO_4 (VWR Chemicals, Radnor, Pennsylvania, USA). BPB was found to have negligible chlorine demand in comparison to the demand exerted by the bacterial spike.

3.4.4 Chlorine disinfection

Chlorine stock solutions

Sodium hypochlorite (NaOCl) stock solutions were prepared daily by diluting laboratory-grade bleach of approximately 10.3% NaOCl (Javex 12 Bleach, The Clorox Company, Oakland, CA, USA) with MilliQ water to reach a FC concentration of approximately 250 mg/L. NaOCl stock solution strength was checked by iodometric titration method (Method 8209, Hach Company, Colorado, USA)[139]. Chlorine stock solution was dosed into the 2 L test flasks at a target FC concentration of either 0.1 or 0.5 mg/L (Figure 3.1). Total Chlorine (TC) and FC were measured using a calibrated colorimeter (DR900, Hach Company, Colorado, USA) and DPD instrument-grade powder pillows (FC: Method 8021; TC: Method 8167, Hach Company, Colorado, USA)[140,141]. All FC and TC measurements were taken in duplicate.

Chlorine exposure protocol

An outline of one FC dose-response run can be found in Figure 3.1. After the test flasks were prepared with spike bacteria, just prior to the addition of NaOCl stock solution (i.e., just before $t = 0$), a small volume of test water was withdrawn and enumerated for *E. coli* as described above. At this time, the pH (pH probe IntelliCAL PHC101; multimeter MQ440D benchtop, both of the Hach Company, Loveland, Colorado, USA) and turbidity were also measured (2020WE turbidimeter, LaMotte, Chestertown, ML, USA), each in duplicate. At the start of each run ($t = 0$), volumes of NaOCl stock solution were added to according to the target FC concentration for the respective run (either 0.1 or 0.5 mg/L, or for control runs, not added), and the 2 L flask was set to stir at lowest speed. After 2, 5 and 10 minutes, approximately 300 mL of test water was withdrawn and quenched with 10 mL of freshly prepared sterile 0.1% sodium sulfite (Na_2SO_3, Caledon Laboratory Chemicals, Toronto, ON, Canada), which is not inhibitory to *E. coli*[142]. At 2 and 10 minutes, a further 100 mL was withdrawn for FC and TC measurement. At the end of the run (after $t = 10$ minutes), the pH was re-measured in duplicate and all quenched samples were enumerated for *E. coli*.

3.4.5 Collimated beam apparatus & UV exposure

Bacterial suspensions were irradiated with a low-pressure (LP), monochromatic collimated beam apparatus (Calgon Carbon UV Technologies LLC, Pittsburgh, PA, USA), which consisted of a 40-Watt low-pressure Mercury-arc lamp (part number A100359, Calgon Carbon UV Technologies LLC, Pittsburgh, PA, USA) emitting nearly monochromatic UV-C radiation at 253.7 nm. The lamp was suspended horizontally within an enclosed box unit directly above the benchtop surface, with a collimating tube leading from an opening in the box to just above the benchtop surface, for a total distance of 56.5 cm from the lamp to the top of the water surface. The collimated beam unit was set up and operated according to best practice outlined by Bolton and Linden[143]. Irradiance was measured at the water surface with an ILT1400 radiometer and SEL240 detector, both calibrated within a year of the experiments (both radiometer and detector from International Light Technologies, Peabody, MS, USA). Average UV dose delivered to the bacterial suspension was determined using the protocol of Bolton et al.[144], accounting for reflection of light off of the water surface, UV absorption by the suspension, distance of the water sample from the UV lamp, and the uniformity of the light distribution across the surface of the sample – the reflection factor, the water factor, the divergence factor and the petri factor, respectively[143,144]. For full instructions on average UV dose calculation including detailed explanation of these four factors, see Bolton and Linden[143] and Bolton et al.[144]; see Table 3.1 for a summary of calculated correction factors. UV absorbance of the test water at the 254 nm wavelength (UVA_{254}) was measured with a spectrophotometer (NanoPhotometer UV/Vis, Implemen GmbH, Munich, Germany). The petri factor was taken daily, and the UV irradiance before and each trial was measured by placing the detector at the sample centerline at the same level as the water surface of irradiated samples. Petri factors ranged from 0.9270 to 0.9968, higher than the minimum of 0.9 recommended by Bolton et al.[144].

Table 3.1: Summary of the correction factors applied to calculate average UV dose for collimated beam experiments, per Bolton et al.[144]

Experimental condition	Correction factor			
	Reflection factor	Water factor	Divergence factor	Petri factor
EcK12 in stationary phase	0.9750	0.9994	0.9928	0.9785
EcN in stationary phase	0.9750	0.9965	0.9928	0.9812
EcN from powder capsule	0.9750	0.9831	0.9928	0.9720

Irradiations were conducted at room temperature; see Figure 3.2 for an outline of the experimental design. For one UV dose-response run, an aliquot of bacterial suspension was drawn from the bulk test water, which was enumerated in advance (preparation described in Section 3.4.2). Aliquots of bacterial suspensions were contained in 35 mm diameter petri dishes (3.5 mL volume, 0.41 cm water path length; petri dishes from Avantor Inc., Radnor, PA, USA). The aliquot was placed on a stir plate directly beneath the collimated beam, such that the aliquot was at the same location and height as the radiometer detector during preliminary petri factor measurements[144]. After the stir plate was set to minimum speed (500 rpm), the timed shutter was opened to exposure the aliquot to the UV collimated beam for a given length of time corresponding to the target UV dose[144] (see Table 3.2). Control runs were conducted by stirring petri dishes beneath the unopened shutter for the median exposure time (i.e., 1 minute and 30 seconds). After UV exposure (or non-exposure, as was the case for control runs), all bacterial samples were serially diluted as appropriate and enumerated for *E. coli* immediately (see Section 3.4.2) to prevent potential dark repair.

Table 3.2: Exposure time corresponding to the target UV dose[144]

Target UV dose, mJ/cm^2	Exposure time, mm:ss
0	00:00
20	00:44
30	01:06
40	01:28
50	01:50
60	02:12
70	02:34

3.4.6 Statistical methods

Once collected, data were entered into Microsoft Excel and analysed using R (version 4.2.1) with Rstudio (version 2022.07.2, build 576), with the Tidyverse (version 1.3.2), Emmeans (version 1.8.6), Rstatix (version 0.7.0) and Readxl (version 1.4.1) libraries. Arithmetic means and 95% confidence intervals (CIs) were calculated for grouped data where applicable (i.e., water quality parameters). Non-detect membrane filtration results were assumed to be one-half the limit of detection (i.e., if 100 mL of water was filtered for membrane filtration, and no *E. coli* colonies were detected, the assumed concentration was 0.5 CFU/100 mL).

A linear regression model was applied to dose-response datasets under each experimental condition following Rennecker-Mariñas[145] (Equation 3.1, adapted to base 10). The parameters N_0 and N are the number of microorganisms before and after the specified $Dose$ (i.e., IT or CT), respectively; k is the inactivation rate constant (also called the Chick-Watson coefficient of lethality per Chick[146] and Watson[147]), or the slope of the linear relationship, and b is the lag coefficient, or the dose below which the microorganisms do not exhibit significant inactivation[51]. For FC dose-response analysis, *E. coli* concentrations taken at $t < 0$ (i.e., prior to NaOCl addition) were excluded from the linear model to improve overall model fit[51], and all other dose-response data points were included (i.e., starting at the lowest dose of 0.1 mg $\cdot$ min/L and upwards). This was due to the substantial jump in LRV from $t < 0$ to $t = 2$ minutes, followed by the declining LRV rate with additional contact time (see Section 3.5). Any observed FC demand that occurred between $t = 2$ minutes and $t = 10$ minutes was assumed to be linear with respect to time, and

chlorine $Dose$ (i.e., CT values) were calculated by integrating the linear decay (i.e., calculating the area beneath the curve) between 2 minutes and 10 minutes[51].

Equation 3.1: The Rennecker-Mariñas model[145] for the dose-response of an organism to disinfection.

$$log_{10}\left({}^{N}/_{N_0}\right) = \begin{cases} 0 & \text{for dose} < b \\ k \cdot (Dose - b) & \text{for dose } \geq b \end{cases}$$

Comparisons between regression models under differing experimental conditions were made using multiple linear regression, with parameter differences considered statistically significant at the $p = 0.05$ confidence level. Where multiple pairwise comparisons were made, the *post-hoc* Bonferroni correction was used to account for increased Type II error.

3.5 Results

3.5.1 Chlorine

The results of FC dose-response testing are displayed in Figure 3.3; model parameters are tabulated in Table 3.3. All three experimental conditions followed a similar pattern: there was a substantial "jump" in LRV (i.e., high inactivation rate) between the zero-dose control and the next lowest CT value, followed by gradual or non-significant inactivation as CT progressed. Reduction of *E. coli* under control conditions was not significant (not shown). Raw data for FC dose-response experiments is available in the SI Table S3.5.

Under the lowest experimental CT dose studied (i.e., a target FC of 0.1 mg/L with a 2-minute contact time), observed mean LRVs were 4.78 (95% CI 3.94; 5.63), 5.65 (95% CI 5.33; 5.97) and 2.72 (95% CI 2.42; 3.02) for EcK12 in stationary phase, EcN in stationary phase, and EcN from powder capsule, respectively (Table 3.3). The observed mean LRVs at the lowest experimental dose were not statistically different between EcN and EcK12 in stationary phase ($p = 0.22$), but EcN dissolved from powder capsule differed significantly between EcN and EcK12 in stationary phase ($p < 0.01$ for both comparisons).

Only EcK12 showed a statistically significant inactivation rate constant k (i.e., different from that of a horizontal line; 0.37 L/mg/min; 95% CI 0.58; 1.5 L/mg/min, see Table 3.3). However, the increased inactivation following the lowest CT was not meaningful; between the lowest and highest experimental CT doses, EcK12 showed an average of 1.69 additional $\log_{10}$ reduction, in comparison to the average 4.79 $\log_{10}$ reduction observed between the zero-dose control and the lowest CT value (Figure 3.3). For EcK12 and EcN in stationary

phase, the dose required for 6 LRV was only somewhat higher than the lowest experimental dose of 0.1 mg · min/L (Table 3.3), meaning that a significant portion of the 6 LRV was achieved as a result of a lowest experimental dose. To achieve 6 LRV for EcN from a powder capsule, a higher FC dose would be required (2.9 mg · min/L; 95% CI 0.5; 5.2 mg · min/L); although this may be due to limited information from censored results under higher FC doses (discussed below).

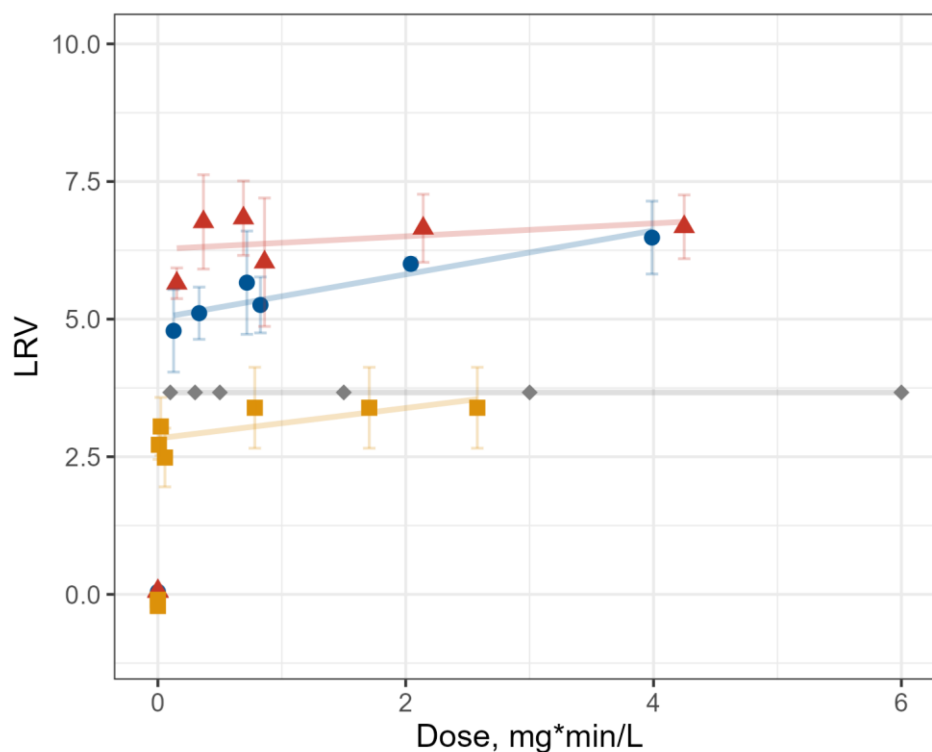

Figure 3.3: The dose-response of E. coli *to free chlorine disinfection.* *EcK12 in stationary phase;* *EcN in stationary phase;* *EcN dissolved directly from powder capsule;* *results reported by Butterfield et al.*[137] *for comparison, at pH 7.0 and 20° to 25°C and 0.1 mg/L target FC. Each point from our experiments represents the arithmetic mean LRV of triplicate runs; error bars represent one standard deviation.*

EcN (both at stationary phase and dissolved directly from powder capsule) did not have statistically meaningful inactivation between the lowest and highest CT values. Both experimental conditions resulted in relatively low R^2 and Pearson's rho values (Table 3.3), with k values that were statistically indistinguishable from a horizontal line (p-value on Pearson's correlation coefficient $p = 0.34$ and $p = 0.09$ for EcN at stationary phase and dissolved directly from powder capsule, respectively). Between the enumeration at $t < 0$

and the lowest CT exposure, EcN in stationary phase exhibited a mean LRV of 5.65, while between the lowest and highest CT exposures, it exhibited an additional 1.05 $\log_{10}$ reduction. When dissolved directly from the powder capsule, EcN had a mean LRV of 2.72 at $t < 0$; between the the lowest and highest CT exposures an additional 0.68 $\log_{10}$ reduction was observed (Figure 3.3). It should be noted that for this condition, under the three highest CT exposures, non-detect values were observed for all runs, i.e., FC exposure reduced EcN to below detectable levels (i.e., < 1 CFU/100 mL). In this case, the resulting LRVs are "right censored", meaning that the "true" LRV could be higher had a higher bacterial spike been used.

Bacterial suspensions under stationary phase (both EcN and EcK12) had a relatively low turbidity ($\bar{X} = 0.06$ NTU; 95% CI 0.05; 0.07 NTU for both strains). Higher turbidity was observed for dissolved EcN runs ($\bar{X} = 5.92$ NTU; 95% CI 5.66; 6.19 NTU), which could create FC demand within the test matrix.

When comparing mean differences in measured FC concentrations between 2 minutes and 10 minutes of contact time, the confidence interval included zero for most run conditions; therefore, the decrease in FC was observed to be negligible. The exception to this were runs where EcN was dissolved from a powder capsule, at a target FC concentration of 0.5 mg/L. The mean difference in FC concentration between measurements between 2 minutes and 10 minutes of contact time was 0.27 mg/L (95% CI 0.20 – 0.33 mg/L), a substantial drop in FC levels. At target FC concentrations of 0.1 mg/L, the FC concentrations dropped to non-detectable levels at the measurement time of 2 minutes, likely due to high chlorine demand in the test water.

3.5.2 UV

The results for *E. coli* inactivation by UV collimated beam are shown in Figure 3.4; curve fitting parameters are shown in Table 3.4. Raw data for FC dose-response experiments is available in the SI Table S3.6. Reduction of *E. coli* under control conditions was not significant (not shown). All three test conditions show relatively high R^2 and Pearson's ρ values and therefore a high degree of correlation. Bacterial suspensions prepared by direct dissolution of the probiotic powder capsule had a higher turbidity ($\bar{X} = 28.3$ NTU, 95% CI 27.5; 29.1 NTU) than those prepared using bacteria in stationary phase washed from TSA ($\bar{X} = 0.23$ NTU, 95% CI 0.21; 0.26 NTU; $\bar{X} = 0.29$ NTU, 95% CI 0.23; 0.34 NTU for EcK12 and EcN, respectively). Bacterial suspensions under all conditions had low UVA_{254}

values, with $2.5 \cdot 10^{-3}$ (95% CI $2.0 \cdot 10^{-3}$; $3.1 \cdot 10^{-3}$), $4.3 \cdot 10^{-3}$ (95% CI $3.3 \cdot 10^{-3}$; $5.2 \cdot 10^{-3}$), and $3.5 \cdot 10^{-2}$ (95% CI $3.4 \cdot 10^{-2}$; $3.5 \cdot 10^{-2}$) for EcK12 in stationary phase, EcN in stationary phase, and EcN dissolved from powder capsule, respectively. EcN shows statistically indistinguishable dose-response behaviour regardless of preparation method (i.e., grown to stationary phase or dissolved from capsule; $p = 0.62$); EcN and EcK12 prepared to stationary phase had statistically homogenous $\boldsymbol{k}$ ($p = 0.59$). However, EcK12 prepared to stationary phase and EcN dissolved from powder capsule had statistically differing $\boldsymbol{k}$ ($p = 0.04$). Reduction of *E. coli* under control conditions was not significant (not shown).

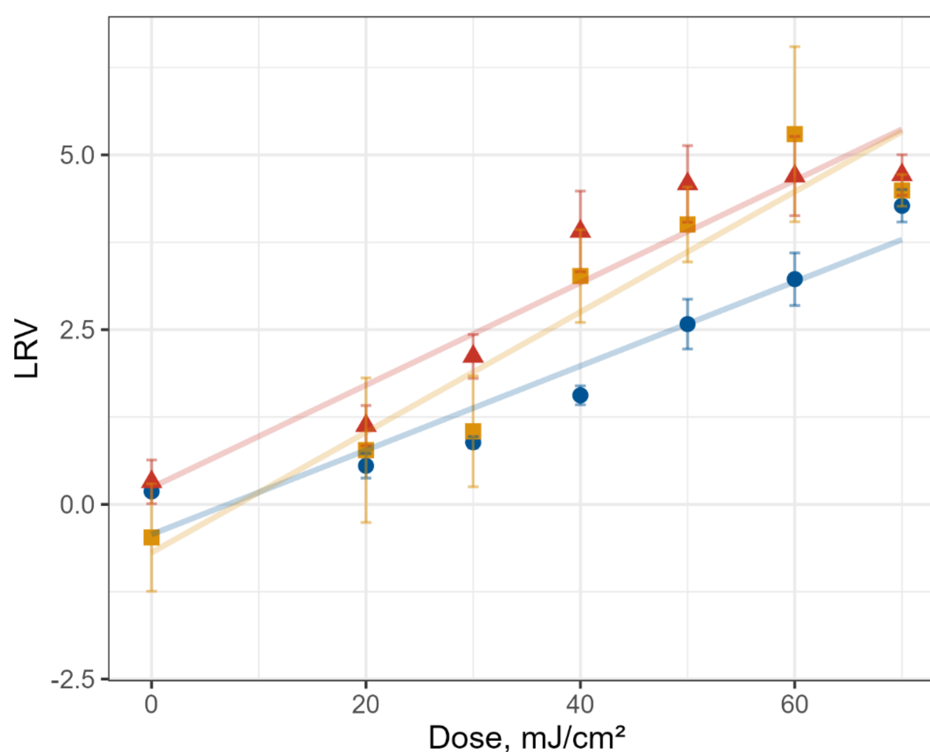

Figure 3.4: The dose-response of E. coli *to UV disinfection.* —●— *EcK12 in stationary phase;* —▲— *EcN in stationary phase;* —■— *EcN dissolved directly from powder capsule. Each point represents the arithmetic mean LRV of triplicate runs; error bars represent standard deviation.*

Table 3.3: Linear modelling parameters and statistical comparisons for chlorine disinfection.

Experimental condition	Linear model parameters					Statistical comparisons between $\boldsymbol{k}$ under each condition
	R^2	Pearson's rho, ρ	Dose required for 6 LRV (95% CI), mg · min/L	LRV at a dose of 0.1 mg · min/L (95% CI)	$\boldsymbol{k}$ [a] (95% CI), L/mg/min	
EcK12 in stationary phase	0.41	0.64	1.8 (1.2; 2.5)	4.79 (3.94; 5.64)	0.37 (0.58;1.5)	EcK12 vs EcN stationary: $p = 0.43$
EcN in stationary phase	0.06	0.24	1.2 (0.4; 2.1)	5.65 (5.33; 5.97)	0.13 (-0.13; 0.38)	EcN stationary vs EcN powder: $p = 0.99$
EcN from powder capsule	0.17	0.41	2.9 (0.5; 5.2)	2.72 (2.42; 3.02)	0.25 (-0.02; 0.52)	EcK12 vs EcN powder: $p = 0.99$

[a] ***k*** *is the inactivation rate constant (also called the Chick-Watson coefficient of lethality per Chick[146] and Watson[147]), or the slope of the linear dose-response relationship.*

Table 3.4: Linear modelling parameters and statistical comparisons for UV disinfection.

Experimental condition	Linear model parameters					Statistical comparisons between $\boldsymbol{k}$ under each condition
	R^2	Pearson's rho, ρ	Dose required for 3 LRV (95% CI), mJ/cm^2	Lag coefficient b [b] (95% CI), mJ/cm^2	$\boldsymbol{k}$ [a] (95% CI), $\cdot 10^{-2}$ cm^2/mJ	
EcK12 in stationary phase	0.90	0.95	55.2 (51.0; 59.4)	10.1 (4.6; 15.7)	6.0 (4.6; 7.4)	EcK12 vs EcN stationary: $p = 0.59$
EcN in stationary phase	0.85	0.92	37.4 (33.6; 41.2)	2.2 (-5.6; 10.0)	7.3 (5.9; 8.8)	EcN stationary vs EcN powder: $p = 0.62$
EcN from powder capsule	0.81	0.90	42.1 (37.5; 46.7)	13.6 (6.3; 20.8)	8.6 (7.2; 10.0)	EcK12 vs EcN powder: $p = 0.04$

[a] ***k*** *is the inactivation rate constant (also called the Chick-Watson coefficient of lethality per Chick[146] and Watson[147]), or the slope of the linear dose-response relationship.*

[b] ***b*** *is the lag coefficient, or the dose below which the microorganisms do not exhibit significant inactivation[51].*

3.6 Discussion

3.6.1 Chlorine

Comparison of EcN and EcK12

Decelerating disinfection (i.e., diminishing LRVs) was observed under all experimental conditions for FC disinfection (Figure 3.3) with respect to time, with low or non-significant inactivation rate constants k (Table 3.3). This phenomenon is often observed after several orders of magnitude of inactivation[51]. The decelerating inactivation we observed could be explained by several possible reasons: natural heterogeneity in disinfection resistance among organisms[148,149]; clumping of microbes that are enumerated as one unit but must be inactivated individually[150]; or a decrease in germicidal properties of the disinfecting agent with time[148,151], although the latter is unlikely for our experiments due to the relatively short contact time. The process of washing microbes from a TSA slant, thoroughly mixing and diluting the mixture should minimise clumping[137] and resulting disinfection resistance; however, this is still a possibility, and in the case of powdered EcN, the washing step was not conducted, so it is more possible in that case.

Comparing EcN and EcK12 in stationary phase, both sets of experiments displayed a statistically similar initial "jump" to a relatively high inactivation (i.e., approximately 5 LRV; Table 3.3). Comparing our results from EcN from a powdered capsule to both EcN and EcK12 in stationary phase, the initial "jump" is statistically lower, but the same pattern of decelerating LRVs is observed. The lower initial "jump" could be explained by the lower starting concentration: approximately 10^3 CFU/100 mL for dissolved EcN, compared to approximately 10^7 CFU/100 mL for EcN and K12 in stationary phase. The starting values for dissolved EcN were limited by the number of organisms in the powder capsule, which was lower than the number of organisms able to be grown on a TSA slant. Another explanation for the lower initial "jump" could be the somewhat higher turbidity observed in the test water when the EcN capsule was dissolved ($\bar{X}$ = 5.92 NTU; 95% CI 5.66 – 6.19 NTU), likely owing to other ingredients listed as present in the powder capsule such as maltodextrin, talc and gelatin[152], which would consume the FC leaving less for disinfection. Indeed, a substantial drop in FC concentration (0.27 mg/L; 95% CI 0.20; 0.33 mg/L) was observed between measurement points at 2 minutes and 10 minutes of contact time for runs with a target FC of 0.5 mg/L, leaving a much lower FC towards the end of

the allocated contact time. This is a limitation of the current work and is further discussed in Section 3.6.3. Particles (i.e., > 7 µm in size) have also shown to have a protective shielding effect against chlorine disinfection[153]. We cannot say to what extent the declining FC and/or particle shielding was responsible for the declining LRVs we observed, but a similar pattern of declining LRVs was observed under the other experimental conditions we studied, in which the FC did not meaningfully decline.

Because *E. coli* spp. are relatively easy to disinfect with FC relative to other typical target microorganisms[51], typical CT values used in water treatment are higher than would be required if *E. coli* were the only target microorganism. In a well-operated treatment system, the CT provided for *Giardia lamblia* (*G. lamblia*) or viruses would easily provide high inactivation of *E. coli*[51]. For example, typical FC concentrations in drinking water treatment plant processes range from 0.4 to 1.5 mg/L, with approximately a 12-minute contact time, producing a CT value on the order of 4.8 to 18 mg · min/L[154]. Although drinking water is not ultrapure MilliQ water as used in our experiments, it is typically high-quality at the point of plant chlorine dosage, so we can somewhat contextualize our results. The typical CT values of 4.8 to 18 mg · min/L[154] are larger than the maximum FC dose in our work and based on our general results, it would be reasonable to expect continued decelerating LRVs until non-detect values are reached under higher real-world CT conditions.

If a higher initial concentration were to be achieved by the direct dissolution of EcN powder capsule into test water (i.e., on the order of magnitude of that achieved by incubation to stationary phase, approximately 10^7 CFU/100 mL) it seems reasonable to expect that the same overall disinfection pattern would be seen as observed in our current experimental work (i.e., an initial jump in LRVs followed by a tail of decelerating inactivation until non-detect values are reached). Although added turbidity due to other powder capsule ingredients may inhibit disinfection due to increased FC demand, *E. coli* are disinfected relatively easily and if exposed to real-world CT conditions (i.e., 4.8 to 18 mg · min/L) it is unlikely that the added turbidity would inhibit inactivation at such CT values.

Comparison with literature

Our results indicate that the strains of *E. coli* we studied (EcN and EcK12) are sensitive to FC disinfection, with a sharp drop in the surviving fraction upon initial contact (i.e., before the first sample interval at $t = 2$ minutes) and a "tail" of decelerating inactivation

thereafter (Figure 3.3). This is in general agreement with the literature, which indicates that *E. coli* spp. are sensitive to disinfection with FC under experimental conditions similar to our own.

The formative work by Butterfield et al.[137,155] found a non-detectable level of *E. coli* (strain unspecified) after exposure to 0.1 mg/L FC after one minute of contact time at room temperature and pH 7.0, for an approximate $3\log_{10}$ reduction (visual comparison to our results plotted in Figure 3.3). Blaser et al.[142] reported 3.54 LRV of *E. coli* (ATCC 11229) after a 1-minute exposure to 0.1 mg/L FC at 25°C and pH 6.0. No additional LRVs were reported after an additional 4 minutes of exposure (i.e., 5 minutes in total), a similar "tailing" phenomenon to that observed in the present results. Chauret et al.[156] noted that the *E. coli* O157:H7 they studied was very sensitive to FC disinfection, with CT values of less than 0.13 mg · min/L required to inactivate approximately $4\log_{10}$. This is a similar result to the other studies using non-pathogenic *E. coli*[137,142,155]. Although not conducted under the same conditions as our work, Lund et al.[157] reported 3 LRV of *E. coli* (strain 304) after a 25-second contact time with 0.2 mg/L FC (i.e., a CT of 0.083 mg · min/L), at pH 6.5 and 10°C. At a higher temperature, the required CT would likely have been lower than 0.083 mg · min/L, meaning that the *E. coli* would have been more easily inactivated, because chlorine disinfection proceeds more quickly at higher temperatures[51].

There are a few studies that are incongruous with the results of the present and other studies. At a lower temperature (5°C) but same pH (7.0) as the current work, Rice et al.[158] reported a 1.1 mg · min/L FC dose required for 4.07 LRV disinfection of *E. coli* O157:H7. Although it is likely that a lower CT would be required for the same LRV at a higher temperature comparable to our study[51], this is still a higher CT than found by ours and other studies. Rice et al.[158] also did not observe decelerating LRVs as in ours and others' studies, but rather a linear relationship between LRV and FC exposure. Under similar temperature (23°C) but different pH (10.3) experimental conditions to the current work, Haas et al.[159] reported a 1.17 LRV of *E. coli* (ATCC 11229) following a CT of 0.4 mg · min/L. This is a comparatively high CT required for a relatively low LRV, but the experiments were conducted at high pH, meaning that the hypochlorite ion (OCl^-) would be the dominant species in the equilibrium reaction, which is a relatively ineffective disinfectant compared to HOCl, the dominant species at $pH < 7.5$[51].

In a review of pathogen and indicator inactivation efficacy by FC, including that of *E. coli*, Petterson and Stenström[154] noted that a statistical meta-analysis of existing data was

not possible due to the high variation in experimental conditions and resulting unquantifiable uncertainties. Although we do not endeavour to undertake a review or meta-analysis, we also note the variation in published experimental conditions and corresponding limitations in result comparability, including comparisons between multiple *E. coli* strains. Varying experimental conditions such as temperature, pH, suspension media and chlorine dosage would confound any such comparison[154]. However, the similarities in behaviour we observed between EcN and EcK12 in stationary phase indicate that some *E. coli* strains would react similarly to FC disinfection under the same conditions. Therefore, EcN seems to be a reasonable surrogate for EcK12 in application as a spike microbe in field challenge test studies, so that *in situ* performance of POUWT methods can be ascertained[20].

3.6.2 UV

Comparison of EcN and EcK12

Our results show a linear dose-response relationship in response to exposure to UV disinfection, for all three experimental conditions studied (Figure 3.4). Dissolved EcN and stationary EcK12 are both statistically similar to stationary EcN, although not to each other (Table 3.4); inactivation rate constant k (i.e., linear model slopes) were statistically comparable between all three experimental conditions tested, except between EcK12 in stationary phase and EcN dissolved directly from the powder capsule (Table 3.4). This indicates that EcN and EcK12 respond similarly to UV disinfection when both at stationary phase, although not when EcN is dissolved from capsule. There is no statistical difference between the inactivation rate constants (k) of EcN at stationary phase compared to dissolved directly from the capsule (Table 3.4), indicating that the effects of the experimental preparation steps on EcN disinfection are negligible.

Although their respective inactivation rate constants were statistically different, the required IT to achieve $3\log_{10}$ reduction was similar (i.e., within the same 95% CI) between EcK12 at stationary phase and dissolved EcN, likely due to the non-negligible lag coefficients (b) in both linear models (Table 3.4). In this respect, the two conditions give comparable results, whereas other pairwise comparisons for $3\log_{10}$ reduction (i.e., EcK12 vs EcN at stationary phase; stationary phase EcN vs dissolved EcN) do not fall within the same 95% CI.

The estimated IT values for 3 LRV under each experimental condition differ by approximately 11% to 30% (Table 3.4). This is not outside the realm of differences observed between published values compiled by Masjoudi et al.[160], for the same strains (i.e., EcK12) and reported experimental conditions. For example, compiled estimates for the required IT for 3 LRV differ by approximately 30%, for EcK12 from the IFO and ATCC strain repositories[160]. Indeed, in some cases, reported IT requirements varied by two to three times between individual studies of EcK12[161–163], or different strains of *E. coli*, such as the pathogenic O157:H7[164] and laboratory strain ATCC 8739[165].

The WHO guidelines[19] for evaluating household water treatment options (including UV disinfection methods) suggest the use of *E. coli* B (ATCC 11303) due to its wide availability, however they do allow for other choices of strain and even species of bacteria, including undifferentiated wild-type *E. coli* strains from diluted wastewater. Many different *E. coli* strains are used for IT testing in the literature, exemplified by the extensive tables by Masjoudi et al.[160]. It seems that the relative difference in dose-response activity (i.e., 10-30%) that we observed in the present work is within inter- and intra-strain differences seen in literature. Therefore, although some statistical differences are seen in the results of our work, the practical differences are not any larger than would be the case if comparing any two studies. Based on this, we conclude that EcN is a practical surrogate for EcK12 in application to field challenge testing of UV-based PoU water treatment.

Comparison with literature

Although the results under the three experimental conditions studied were reasonably similar to each other, they differed from what was expected based on the published literature. The expected dose required for $3\log_{10}$ inactivation of EcK12 via UV under similar experimental conditions (i.e., collimated beam apparatus with a standing sample volume) is approximately 6.0 to 9.4 mJ/cm^2 [160]. The modelled UV dose for $3\log_{10}$ inactivation of EcK12 in our study was 55.2 mJ/cm^2 (95% CI 51.0; 59.4 mJ/cm^2), approximately 5.9 to 9.2 times higher than literature estimates. Indeed, we could not find a literature estimate that approached our results.

3.6.3 Study limitations

As our IT estimates are much higher than expected based on literature, but reasonably similar under each experimental condition, we suspect that there may be a systematic error associated with our experimental work. Either the measured dose was much higher than the "true" dose, resulting in an inflated IT requirement, or the measured bacterial reductions were lower than the "true" reductions which would also result in an inflated IT requirement. It is unknown as yet precisely what systematic error has led to the higher-than-expected IT results. However, due to the likely systematic nature of the error, comparisons within our experimental results are still valid. We can still conclude based on this data that EcN dissolved from the capsule behaves comparably to the laboratory strain EcK12, under conditions expected in application to field challenge testing.

Although a range of water quality parameters would be expected if implementing field challenge testing in various settings, for our disinfection experiments, only one type of water (i.e., Butterfield's buffer at pH 7.0) was used. It is therefore unknown what impact fluctuations of water quality would have on the disinfection behaviour of EcN compared to EcK12, for example if the low-quality test water was used per WHO[19], with a high (> 30 NTU) turbidity, low (4°C) temperature and variable (6-10) pH. Particularly for FC, a higher pH would cause the hypochlorous acid-hypochlorite ion ($HOCl \leftrightarrow OCl^-$) equilibrium reaction to favour the hypochlorite ion (OCl^-), which is a significantly weaker disinfectant[51]. A lower temperature would slow FC disinfection[51] although the extent to which either of these factors would impact disinfection of EcN versus EcK12 is unknown. The drop in FC concentrations observed in FC dose-response runs with EcN dissolved directly from a powder capsule (0.27 mg/L; 95% CI 0.20; 0.33 mg/L) could be attributed to the somewhat higher turbidity ($\bar{X}$ = 5.92 NTU; 95% CI 5.66 – 6.19 NTU) resulting from other powder ingredients present in the capsule. This drop in FC concentration was not observed in other experimental conditions in which *E. coli* spp. were grown from stationary phase and is a limitation of the current study. Despite this limitation, the same pattern of behaviour was observed with EcN dissolved directly from a powder capsule compared to EcN and EcK12 grown from stationary phase (i.e., the initial "jump" in LRVs, followed by a "tail" of declining LRVs as dose increases), and therefore it would be reasonable to conclude that most disinfection takes place during this initial "jump", and therefore the declining FC concentration would not impact the overall disinfection behaviour of EcN when directly dissolved from the powder capsule.

With respect to UV disinfection, a higher turbidity could "shield" the microorganisms from the UV light, making them more difficult to disinfect[51], although the extent to which this would impact EcN versus EcK12 is again unknown. In our UV experiments, we only studied disinfection using a low-pressure mercury lamp emitting light at 253.7 nm (see Section 3.4.5), which is potentially dissimilar to some SODIS and UV-LED conditions. UV-LED lamps are available in many point-value wavelengths within the UV range[160], which may result in differing dose-response results between EcN and EcK12. SODIS approaches use sunlight to disinfect water, including but not limited to the UV-A and UV-C ranges, as well as the synergistic effect of temperature to compound UV disinfection[66]. We did not study broad-spectrum UV effects, nor heating effects, although EcN and EcK12 did perform comparably under the conditions studied.

Photoreactivation ("dark repair") occurs in most microbes following UV exposure[166], which can impact the effectiveness of UV disinfection approaches[167–169]. We did not study this phenomenon in the present work; recent analyses show that dark repair should not pose a significant health risk if a relatively low minimum dose is achieved and the water is consumed reasonably promptly[170], as is typical for SODIS[52].

3.7 Conclusion

There have been recent calls to evaluate POUWT methods in ways that are more reflective of real use conditions. To this end, field challenge testing has been proposed in order to assess microbe reduction of PoU treatment methods at the actual point of use, to potentially encapsulate *in situ* treatment performance. To satisfy prospective ethical and safety requirements, a food-safe bacterial surrogate has been identified: a probiotic health supplement containing non-pathogenic *E. coli* Nissle, or EcN, which has not yet been assessed for use with free chlorine or UV disinfection. The goal of the present study was to validate EcN against *E. coli* K-12 (EcK12), a standard laboratory strain, and to assess the change (if any) in disinfection efficacy of EcN arising a simplified preparation method as would be the case under field use conditions.

Dose-response experiments were conducted using either free chlorine or UV disinfection methods. With respect to free chlorine disinfection, EcN and EcK12 responded similarly under all conditions studied, although when EcN was dissolved directly from the powder capsule into test water prior to testing, the low initial *E. coli* concentration limited comparisons due to censored observations (i.e., right-censored LRVs). We observed a sharp

drop in microbe concentrations when exposed to the lowest possible free chlorine dose, with declining LRVs observed with increasing CT.

When exposed to UV disinfection, there were some statistically significant differences in inactivation rate constants and IT required for $3\log_{10}$ reduction between some conditions studied. However, the statistical differences were not substantive when compared to between-study differences in dose-response behaviour for the same *E. coli* strains within the published literature. We therefore conclude that EcN is a practical surrogate for EcK12 in application to field challenge testing of POUWT devices via free chlorine or UV disinfection. By gathering field-relevant data, the influence of the contexts in which POUWT are used can be accurately reflected.

3.8 Author contributions

Conceptualization: CZ and CCD; methodology: CZ, SB and CCD; laboratory experiments: CZ and AG; statistical analysis: CZ; figure preparation: CZ; writing – original draft: CZ; writing – review and editing: CZ and CCD; supervision: CCD.

3.9 Supplementary information

Table S3.5: Raw data from FC dose-response experiments used to determine the Rennecker-Mariñas model parameters[145].

Run name	Run number	Date performed	*E. coli* strain	Preparation method	Free Cl target conc, mg/L	Test solution turbidity, NTU (average of 2-3 consecutive measurements)	Test solution pH before bleach stock solution added	Bleach stock solution Free Cl concentration, mg/L	Vol. bleach stock added to 2 L test solution, mL	Test solution pH at the end of the run	Slope of chlorine Ct line	Intercept of chlorine Ct line	Free chlorine demand, 2 min to 10 min	Influent dilution processed (vial no.)	Influent CFU - A	Influent CFU - B	Influent CFU -C	Influent Concentration, CFU/100 mL
K12_TSA_0.0mg_A	21	2022-07-20	K12	TSA	0	0.07	7.01	0	0	6.96	0.00000	0.00000	0.00000	4	78	67	93	7933333
K12_TSA_0.1mg_A	16	2022-06-07	K12	TSA	0.1	0.05	7.04	234	1	6.95	0.00125	0.05417	-0.01000	4	176	167	94	14566667
K12_TSA_0.1mg_B	6	2022-05-18	K12	TSA	0.1	0.05	6.97	240	1	7.20	0.00500	0.04000	-0.04000	4	95	59	12	5533333
K12_TSA_0.1mg_C	8	2022-05-18	K12	TSA	0.1	0.04	6.93	240	1	7.06	0.00000	0.09000	0.00000	4	50	63	87	6666667
K12_TSA_0.5mg_A	15	2022-05-31	K12	TSA	0.5	0.11	7.05	250	4	7.07	-0.00042	0.36417	0.00333	4	94	66	83	8100000
K12_TSA_0.5mg_B	12	2022-05-27	K12	TSA	0.5	0.07	6.98	240	4	6.95	-0.00708	0.48083	0.05667	4	113	338	144	19833333
K12_TSA_0.5mg_C	9	2022-05-27	K12	TSA	0.5	0.07	7.06	240	4	7.14	-0.00375	0.40750	0.03000	4	44	57	55	5200000
Mutaflor_capsule_0.0mg_A	19	2022-07-19	Mutaflor	capsule	0	6.66	7.04	0	0	6.92	0.00000	0.00000	0.00000	1	17	5	10	1067
Mutaflor_capsule_0.1mg_A	11	2022-05-27	Mutaflor	capsule	0.1	5.02	7.02	240	4	7.00	0.00125	0.01083	-0.01000	2	1	0	0	333
Mutaflor_capsule_0.1mg_B	14	2022-05-31	Mutaflor	capsule	0.1	5.49	6.98	250	4	7.00	0.00000	0.00000	0.00000	1	28	7	17	1733
Mutaflor_capsule_0.1mg_C	1	2022-05-06	Mutaflor	capsule	0.1	5.44	7.05	247	4	NR	0.00000	0.00000	0.00000	2	2	0	7	3000
Mutaflor_capsule_0.5mg_A	10	2022-05-27	Mutaflor	capsule	0.5	5.90	7.05	240	10	7.15	-0.02500	0.31000	0.20000	2	2	0	0	667
Mutaflor_capsule_0.5mg_B	2	2022-05-06	Mutaflor	capsule	0.5	6.22	6.98	247	10	NR	-0.03875	0.45750	0.31000	2	10	10	5	8333
Mutaflor_capsule_0.5mg_C	5	2022-05-10	Mutaflor	capsule	0.5	6.75	7.04	252	10	7.12	-0.03625	0.50583	0.29000	2	0	1	0	333
Mutaflor_TSA_0.0mg_A	20	2022-07-19	Mutaflor	TSA	0	0.06	6.97	0	0	6.93	0.00000	0.00000	0.00000	4	86	81	74	8033333
Mutaflor_TSA_0.1mg_A	3	2022-05-10	Mutaflor	TSA	0.1	0.07	7.04	252	1	7.18	-0.00375	0.10750	0.03000	4	86	205	80	12366667
Mutaflor_TSA_0.1mg_B	17	2022-06-07	Mutaflor	TSA	0.1	0.10	6.97	234	1	6.83	-0.00125	0.06250	0.01000	4	241	249	208	23266667
Mutaflor_TSA_0.1mg_C	18	2022-06-07	Mutaflor	TSA	0.1	0.05	6.97	234	1	6.95	-0.00042	0.06417	0.00333	4	532	588	584	56800000
Mutaflor_TSA_0.5mg_A	13	2022-05-31	Mutaflor	TSA	0.5	0.08	7.04	250	4	7.00	-0.00167	0.40333	0.01333	4	225	235	252	23733333
Mutaflor_TSA_0.5mg_B	4	2022-05-10	Mutaflor	TSA	0.5	0.05	7.06	252	4	7.12	0.00000	0.46667	0.00000	4	92	76	111	9300000
Mutaflor_TSA_0.5mg_C	7	2022-05-18	Mutaflor	TSA	0.5	0.04	7.04	240	4	7.07	-0.00250	0.42500	0.02000	4	5	50	204	8633333

Run name	2 minute Free Cl concentration, mg/L (average of 2-3 consecutive measurements)	2 minute Total Cl concentration, mg/L (average of 2-3 consecutive measurements)	2 minute Ct, mg/L*min (free Cl), using slope of a straight line	2 minute effluent volume, mL	2 minute effluent CFU - A	2 minute effluent CFU - B	2 minute effluent CFU - C	2 minute effluent concentration, CFU/100 mL *0 changed to 0.5	2 min censored (Y/N)	2 minute LRV
K12_TSA_0.0mg_A	0.00	0.01	0.000	0.001	76	87	74	7900000.0	N	0.00
K12_TSA_0.1mg_A	0.06	0.10	0.111	100	21	63	13	32.3	N	5.65
K12_TSA_0.1mg_B	0.05	0.11	0.090	100	158	440	176	258.0	N	4.33
K12_TSA_0.1mg_C	0.09	0.12	0.180	100	274	280	281	278.3	N	4.38
K12_TSA_0.5mg_A	0.36	0.46	0.728	100	6	15	23	14.7	N	5.74
K12_TSA_0.5mg_B	0.47	0.49	0.948	100	99	85	113	99.0	N	5.30
K12_TSA_0.5mg_C	0.40	0.46	0.808	100	43	80	168	97.0	N	4.73
Mutaflor_capsule_0.0mg_A	0.00	0.00	0.000	1	9	25	18	1733.3	N	-0.21
Mutaflor_capsule_0.1mg_A	0.01	0.26	0.024	100	0	0	0	0.5	Y	2.82
Mutaflor_capsule_0.1mg_B	0.00	0.27	0.000	100	9	6	5	6.7	N	2.41
Mutaflor_capsule_0.1mg_C	0.00	0.31	0.000	100	1	10	0	3.7	N	2.91
Mutaflor_capsule_0.5mg_A	0.26	0.78	0.570	100	0	0	0	0.5	Y	3.12
Mutaflor_capsule_0.5mg_B	0.38	0.88	0.838	100	0	1	0	0.5	Y	4.22
Mutaflor_capsule_0.5mg_C	0.43	0.83	0.939	100	0	0	0	0.5	Y	2.82
Mutaflor_TSA_0.0mg_A	0.00	0.00	0.000	0.001		72	69	7050000.0	N	0.06
Mutaflor_TSA_0.1mg_A	0.10	0.11	0.208	100	17	8	16	13.7	N	5.96
Mutaflor_TSA_0.1mg_B	0.06	0.11	0.123	100	68	54	58	60.0	N	5.59
Mutaflor_TSA_0.1mg_C	0.06	0.11	0.128	100	212	201	253	222.0	N	5.41
Mutaflor_TSA_0.5mg_A	0.40	0.46	0.803	100	0	3	1	1.3	N	7.25
Mutaflor_TSA_0.5mg_B	0.47	0.50	0.933	100	11	8	14	11.0	N	5.93
Mutaflor_TSA_0.5mg_C	0.42	0.47	0.845	100	75	87	146	102.7	N	4.92

Run name	5 minute Ct, mg/L*min (free Cl), using slope of a straight line	5 minute effluent volume, mL	5 minute effluent CFU - A	5 minute effluent CFU - B	5 minute effluent CFU - C	5 minute effluent concentration, CFU/100 mL *0 changed to 0.5	5 min censored (Y/N)	5 minute LRV
K12_TSA_0.0mg_A	0.000	0.001	86	65	63	7133333.333	N	0.05
K12_TSA_0.1mg_A	0.286	100	33	34	37	34.66666667	N	5.62
K12_TSA_0.1mg_B	0.263	100	60	89	190	113	N	4.69
K12_TSA_0.1mg_C	0.450	100	64	83	48	65	N	5.01
K12_TSA_0.5mg_A	1.816	100	7	4	8	6.333333333	N	6.11
K12_TSA_0.5mg_B	2.316	100	39	22	16	25.66666667	N	5.89
K12_TSA_0.5mg_C	1.991	100	3	3	9	5	N	6.02
Mutaflor_capsule_0.0mg_A	0.000	1	2	18	20	1333.333333	N	-0.10
Mutaflor_capsule_0.1mg_A	0.070	100	0	0	0	0.5	Y	2.82
Mutaflor_capsule_0.1mg_B	0.000	100	2	8	1	3.666666667	N	2.67
Mutaflor_capsule_0.1mg_C	0.000	100	0	0	2	0.666666667	N	3.65
Mutaflor_capsule_0.5mg_A	1.238	100	0	0	0	0.5	Y	3.12
Mutaflor_capsule_0.5mg_B	1.803	100	0	0	0	0.5	Y	4.22
Mutaflor_capsule_0.5mg_C	2.076	100	0	0	0	0.5	Y	2.82
Mutaflor_TSA_0.0mg_A	0.000	0.001	73	78	64	7166666.667	N	0.05
Mutaflor_TSA_0.1mg_A	0.491	100	6	8	4	6	N	6.31
Mutaflor_TSA_0.1mg_B	0.297	100	17	16	8	13.66666667	N	6.23
Mutaflor_TSA_0.1mg_C	0.316	100	1	0	2	1	N	7.75
Mutaflor_TSA_0.5mg_A	1.996	100	3	13	0	5.333333333	N	6.65
Mutaflor_TSA_0.5mg_B	2.333	100	0	0	0	0.5	Y	7.27
Mutaflor_TSA_0.5mg_C	2.094	100	9	7	8	8	N	6.03

Run name	10 minute Free Cl concentration, mg/L (average of 2-3 consecutive measurements)	10 minute Total Cl concentration, mg/L (average of 2-3 consecutive measurements)	10 minute Ct, mg/L*min (free Cl), using slope of a straight line	10 minute effluent volume, mL	10 minute effluent CFU - A	10 minute effluent CFU - B	10 minute effluent CFU - C	10 minute effluent concentration, CFU/100 mL *0 changed to 0.5	10 min censored (Y/N)	10 minute LRV
K12_TSA_0.0mg_A	0.00	0.00	0.000	0.001	91	80	70	8033333.3	N	-0.01
K12_TSA_0.1mg_A	0.07	0.10	0.604	100	1	4	3	2.7	N	6.74
K12_TSA_0.1mg_B	0.09	0.10	0.650	100	26	26	43	31.7	N	5.24
K12_TSA_0.1mg_C	0.09	0.12	0.900	100	71	71	54	65.3	N	5.01
K12_TSA_0.5mg_A	0.36	0.47	3.621	100	0	2	3	1.7	N	6.69
K12_TSA_0.5mg_B	0.41	0.46	4.454	100	35	38	35	36.0	N	5.74
K12_TSA_0.5mg_C	0.37	0.44	3.888	100	0	0	0	0.5	Y	7.02
Mutaflor_capsule_0.0mg_A	0.00	0.00	0.000	1	23	12	16	1700.0	N	-0.20
Mutaflor_capsule_0.1mg_A	0.02	0.26	0.171	100	0	0	0	0.5	Y	2.82
Mutaflor_capsule_0.1mg_B	0.00	0.27	0.000	100	3	1	5	3.0	N	2.76
Mutaflor_capsule_0.1mg_C	0.00	0.30	0.000	100	1	120	0	40.3	N	1.87
Mutaflor_capsule_0.5mg_A	0.06	0.70	1.850	100	0	0	0	0.5	Y	3.12
Mutaflor_capsule_0.5mg_B	0.07	0.79	2.638	100	0	0	0	0.5	Y	4.22
Mutaflor_capsule_0.5mg_C	0.14	0.84	3.246	100	0	0	0	0.5	Y	2.82
Mutaflor_TSA_0.0mg_A	0.00	0.00	0.000	0.001	60	76	72	6933333.3	N	0.06
Mutaflor_TSA_0.1mg_A	0.07	0.11	0.888	100	3	3	0	2.0	N	6.79
Mutaflor_TSA_0.1mg_B	0.05	0.11	0.563	100	11	13	22	15.3	N	6.18
Mutaflor_TSA_0.1mg_C	0.06	0.11	0.621	100	0	3	2	1.7	N	7.53
Mutaflor_TSA_0.5mg_A	0.39	0.45	3.950	100	1	8	7	5.3	N	6.65
Mutaflor_TSA_0.5mg_B	0.47	0.48	4.667	100	0	0	0	0.5	Y	7.27
Mutaflor_TSA_0.5mg_C	0.40	0.48	4.125	100	10	6	4	6.7	N	6.11

Table S3.6: Raw data from UV dose-response experiments used to determine the Rennecker-Mariñas model parameters[145].

Run name	Date	*E. coli* strain	Prep method	pH	Turbidity	UVA 254	Petri factor	Influent dilution processed (vial no.)	Influent CFU - A	Influent CFU - B	Influent CFU -C	Dose (mJ/cm2)	Exposure time (min:sec)	Effluent dilution processed (vial no.)	Effluent effluent CFU - A	Effluent effluent CFU - B	Effluent effluent CFU - C
K_TSA_day3	10-Jan-23	K12	TSA	7.0	0.2	0.0010	0.9703	5	27	39	32	30	1:05	4	58	49	50
K_TSA_day3	10-Jan-23	K12	TSA	7.0	0.2	0.0010	0.9703	5	27	39	32	40	1:27	3	110	100	120
K_TSA_day3	10-Jan-23	K12	TSA	7.0	0.2	0.0010	0.9703	5	27	39	32	60	2:10	2	41	55	53
M_cap_day2	12-Jan-23	Mutaflor	Capsule	7.0	29.5	0.0320	0.9270	5	59	69	85	20	0:45	3	760	1032	888
M_cap_day2	12-Jan-23	Mutaflor	Capsule	7.0	29.5	0.0320	0.9270	5	59	69	85	30	1:08	3	222	215	202
M_cap_day2	12-Jan-23	Mutaflor	Capsule	7.0	29.5	0.0320	0.9270	5	59	69	85	40	1:30	3	3	3	5
M_cap_day2	12-Jan-23	Mutaflor	Capsule	7.0	29.5	0.0320	0.9270	5	59	69	85	50	1:53	2	2	2	2
M_cap_day2	12-Jan-23	Mutaflor	Capsule	7.0	29.5	0.0320	0.9270	5	59	69	85	50	1:53	2	10	6	6
M_cap_day2	12-Jan-23	Mutaflor	Capsule	7.0	29.5	0.0320	0.9270	5	59	69	85	70	2:38	2	1	0	4
M_TSA_day3	17-Jan-23	Mutaflor	TSA	6.9	0.1	0.0075	0.9812	5	68	72	78	20	0:44	4	78	92	79
M_TSA_day3	17-Jan-23	Mutaflor	TSA	6.9	0.1	0.0075	0.9812	5	68	72	78	20	0:44	4	73	84	72
M_TSA_day3	17-Jan-23	Mutaflor	TSA	6.9	0.1	0.0075	0.9812	5	68	72	78	40	1:28	3	2	1	1
M_TSA_day3	17-Jan-23	Mutaflor	TSA	6.9	0.1	0.0075	0.9812	5	68	72	78	40	1:28	3	2	2	3
M_TSA_day3	17-Jan-23	Mutaflor	TSA	6.9	0.1	0.0075	0.9812	5	68	72	78	60	2:12	2	0	1	18
M_TSA_day3	17-Jan-23	Mutaflor	TSA	6.9	0.1	0.0075	0.9812	5	68	72	78	70	2:34	1	2	1	42
M_TSA_day3	17-Jan-23	Mutaflor	TSA	6.9	0.1	0.0075	0.9812	5	68	72	78	70	2:34	1	3	3	15
K_TSA_day1	24-Jan-23	K12	TSA	6.9	0.2	0.0013	0.9785	5	163	140	149	0	0:45	5	107	94	101
K_TSA_day1	24-Jan-23	K12	TSA	6.9	0.2	0.0013	0.9785	5	163	140	149	0	1:52	5	101	93	103
K_TSA_day1	24-Jan-23	K12	TSA	6.9	0.2	0.0013	0.9785	5	163	140	149	20	0:45	4	262	287	248
K_TSA_day1	24-Jan-23	K12	TSA	6.9	0.2	0.0013	0.9785	5	163	140	149	50	1:52	2	288	258	292
K_TSA_day1	24-Jan-23	K12	TSA	6.9	0.2	0.0013	0.9785	5	163	140	149	50	1:52	2	245	201	218
K_TSA_day1	24-Jan-23	K12	TSA	6.9	0.2	0.0013	0.9785	5	163	140	149	60	2:15	2	43	40	41
K_TSA_day1	24-Jan-23	K12	TSA	6.9	0.2	0.0013	0.9785	5	163	140	149	70	2:37	1	60	56	77
M_TSA_day1	31-Jan-23	Mutaflor	TSA	7.0	0.5	0.0030	0.9577	5	422	324	400	0	0:45	5	112	134	133
M_TSA_day1	31-Jan-23	Mutaflor	TSA	7.0	0.5	0.0030	0.9577	5	422	324	400	0	1:53	5	101	107	135
M_TSA_day1	31-Jan-23	Mutaflor	TSA	7.0	0.5	0.0030	0.9577	5	422	324	400	20	0:45	4	119	145	135
M_TSA_day1	31-Jan-23	Mutaflor	TSA	7.0	0.5	0.0030	0.9577	5	422	324	400	30	1:08	4	14	13	11
M_TSA_day1	31-Jan-23	Mutaflor	TSA	7.0	0.5	0.0030	0.9577	5	422	324	400	40	1:30	3	0	1	1
M_TSA_day1	31-Jan-23	Mutaflor	TSA	7.0	0.5	0.0030	0.9577	5	422	324	400	50	1:53	2	0	11	58
M_TSA_day1	31-Jan-23	Mutaflor	TSA	7.0	0.5	0.0030	0.9577	5	422	324	400	50	1:53	2	1	0	6
M_cap_day3	02-Feb-23	Mutaflor	Capsule	7.1	28.7	0.0375	0.9284	5	43	44	62	20	0:46	4	12	9	6
M_cap_day3	02-Feb-23	Mutaflor	Capsule	7.1	28.7	0.0375	0.9284	5	43	44	62	30	1:09	4	11	26	12
M_cap_day3	02-Feb-23	Mutaflor	Capsule	7.1	28.7	0.0375	0.9284	5	43	44	62	40	1:32	3	8	12	18
M_cap_day3	02-Feb-23	Mutaflor	Capsule	7.1	28.7	0.0375	0.9284	5	43	44	62	70	2:42	1	15	22	51
M_TSA_day2	14-Feb-23	Mutaflor	TSA	7.1	0.3	0.0025	0.9970	5	111	141	142	0	2:14	5	129	157	146
M_TSA_day2	14-Feb-23	Mutaflor	TSA	7.1	0.3	0.0025	0.9970	5	111	141	142	30	1:07	3	153	163	132
M_TSA_day2	14-Feb-23	Mutaflor	TSA	7.1	0.3	0.0025	0.9970	5	111	141	142	30	1:07	3	155	169	145
M_TSA_day2	14-Feb-23	Mutaflor	TSA	7.1	0.3	0.0025	0.9970	5	111	141	142	40	1:29	2	20	22	50
M_TSA_day2	14-Feb-23	Mutaflor	TSA	7.1	0.3	0.0025	0.9970	5	111	141	142	50	1:52	2	0	0	19
M_TSA_day2	14-Feb-23	Mutaflor	TSA	7.1	0.3	0.0025	0.9970	5	111	141	142	60	2:14	1	17	11	24
M_TSA_day2	14-Feb-23	Mutaflor	TSA	7.1	0.3	0.0025	0.9970	5	111	141	142	60	2:14	1	14	9	5
M_TSA_day2	14-Feb-23	Mutaflor	TSA	7.1	0.3	0.0025	0.9970	5	111	141	142	70	2:36	1	32	47	65
M_cap_day1	16-Feb-23	Mutaflor	Capsule	7.0	28.9	0.0343	0.9968	5	24	16	18	0	2:30	5	161	163	152
M_cap_day1	16-Feb-23	Mutaflor	Capsule	7.0	28.9	0.0343	0.9968	5	24	16	18	0	1:47	5	148	167	168
M_cap_day1	16-Feb-23	Mutaflor	Capsule	7.0	28.9	0.0343	0.9968	5	24	16	18	20	0:43	4	390	364	446
M_cap_day1	16-Feb-23	Mutaflor	Capsule	7.0	28.9	0.0343	0.9968	5	24	16	18	30	1:04	4	157	143	132
M_cap_day1	16-Feb-23	Mutaflor	Capsule	7.0	28.9	0.0343	0.9968	5	24	16	18	50	1:47	1	66	46	81
M_cap_day1	16-Feb-23	Mutaflor	Capsule	7.0	28.9	0.0343	0.9968	5	24	16	18	60	2:09	1	5	5	5
M_cap_day1	16-Feb-23	Mutaflor	Capsule	7.0	28.9	0.0343	0.9968	5	24	16	18	60	2:09	1	5	5	6
M_cap_day1	16-Feb-23	Mutaflor	Capsule	7.0	28.9	0.0343	0.9968	5	24	16	18	70	2:30	1	2	1	11
K_TSA_day2	02-Mar-23	K12	TSA	7.0	0.3	0.0040	0.9690	5	110	132	112	0	2:36	5	72	85	67
K_TSA_day2	02-Mar-23	K12	TSA	7.0	0.3	0.0040	0.9690	5	110	132	112	20	0:45	5	48	23	52
K_TSA_day2	02-Mar-23	K12	TSA	7.0	0.3	0.0040	0.9690	5	110	132	112	20	0:45	5	45	45	38
K_TSA_day2	02-Mar-23	K12	TSA	7.0	0.3	0.0040	0.9690	5	110	132	112	30	1:07	4	138	155	138
K_TSA_day2	02-Mar-23	K12	TSA	7.0	0.3	0.0040	0.9690	5	110	132	112	40	1:29	3	364	416	360
K_TSA_day2	02-Mar-23	K12	TSA	7.0	0.3	0.0040	0.9690	5	110	132	112	50	1:51	3	78	75	85
K_TSA_day2	02-Mar-23	K12	TSA	7.0	0.3	0.0040	0.9690	5	110	132	112	60	2:14	2	66	69	48
K_TSA_day2	02-Mar-23	K12	TSA	7.0	0.3	0.0040	0.9690	5	110	132	112	70	2:36	1	30	35	64
M_cap_day4	03-Mar-23	Mutaflor	Capsule	7.1	24.0	0.0365	0.9720	4	38	22	23	0	0:44	4	12	12	8
K_TSA_day4	03-Mar-23	K12	TSA	6.9	0.3	0.0030	0.9720	5	75	52	49	30	1:05	4	59	56	82
K_TSA_day4	03-Mar-23	K12	TSA	6.9	0.3	0.0030	0.9720	5	75	52	49	40	1:27	3	122	127	90
M_cap_day4	03-Mar-23	Mutaflor	Capsule	7.1	24.0	0.0365	0.9720	4	38	22	23	40	1:28	2	0	0	1
M_cap_day4	03-Mar-23	Mutaflor	Capsule	7.1	24.0	0.0365	0.9720	4	38	22	23	60	2:12	1	0	0	0
K_TSA_day4	03-Mar-23	K12	TSA	6.9	0.3	0.0030	0.9720	5	75	52	49	70	2:32	1	35	56	82

Chapter 4: Assessing the suitability of probiotic supplement EcN for microbiological quality control of ceramic water filters

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4.1 Abstract

Ceramic water filters (CWFs) are an established approach to household water treatment with widespread adoption. There is a need for on-premises or easily accessible microbiological quality control for local CWF manufacturers to produce high-quality filters and therefore increase public health protection and consumer trust. A simplified field-based challenge test protocol has been proposed to be implemented at the manufacturing site, to expand the accessibility of microbiological quality control (QC) testing and more readily identify microbial risk-related filter weaknesses.

The aim of this study was to validate the previously-studied probiotic *E. coli* (strain name Nissle 1917, or EcN, brand name Mutaflor®) against a standard laboratory strain of *E. coli* K-12 (EcK12) in application to filtration challenge testing. To this end, microbiological challenge testing was undertaken in the laboratory with non-silver-coated ceramic “candle”

filters using both EcN and EcK12. To provide context for filtration testing, *E. coli* morphological characteristics of zeta potential and particle size distribution were measured.

Although we found statistically significant albeit modest morphological differences (i.e., size and zeta potential) between EcN and EcK12, both strains of *E. coli* responded similarly to ceramic filtration. Based on our laboratory findings and additional priority surrogate attributes of safety and ease of field quantification, we conclude that EcN is a useful and feasible spike organism to represent the bacterial pathogen class in challenge testing. The anticipated application of EcN is for use as a challenge organism in on-site or decentralized microbiological quality control testing of CWFs, which would decrease some barriers towards more widely adopted microbiological quality control.

4.2 Keywords

Ceramic water filters; challenge test; point of use water treatment; zeta potential.

4.3 Introduction

4.3.1 Background on ceramic water filters

Household water treatment and storage (HWTS) can be a cost-effective way to improve the microbiological quality of drinking water[52,65] and thus reduce the incidence of diarrheal disease for those with limited access to an improved water source[171]. Ceramic water filters (CWFs) are an established approach to household water treatment with widespread adoption – about 50 factories globally[126]. CWFs have been widely evaluated for microbiological effectiveness, originating from factories in Cambodia[172,173], India[174,175], Guatemala[82], Bolivia[176], Mexico[177], China[84], Ghana and Nicaragua[173].

CWFs can be fabricated into different shapes, such as a disk, tube, candle or pot, which make them suitable for point-of-use (PoU) applications in various situations[54]. They are comprised of a ceramic filter element (disk, tube, candle or pot) suspended within a covered reservoir having a 10 – 20 L capacity (e.g., Figure 4.1). CWFs are typically manufactured by pressing locally-sourced clay, water, and ground burn-out material (such as rice husks or sawdust) into shape; once dry, filters are fired into ceramic (approximately 900°C), with burn-out material combusting to leave a porous matrix, allowing for water purification mainly via size exclusion of pathogens and particles[57]. Finished filters can be impregnated

with colloidal silver, which has antimicrobial qualities, to provide further disinfection and prevent biofilm growth on the filter wall[54,57,178].

CWFs have a high potential to be widely and sustainably used[121] and rank highly in terms of effectiveness, cost, ease of use and microbiological protection[54,55], making them a promising household treatment method[59,176,179]. CWFs are typically locally produced at decentralised facilities using locally-available materials[57–59], a production model that lends advantages of logistical simplicity, flexible production, small business opportunities and local supply chain access[180]. CWFs have been estimated to remove up to 99.999% of bacterial organisms from water[121,181,182]. High user satisfaction has been reported with CWFs[182–184] and reduced risk of diarrheal illness is associated with use[176,185]. A relatively high degree of adoption (i.e., consistency of use, per Levy[186]) has been observed with CWFs[24,184], which is an essential component of effective HWTS practice in reducing the risk of illness[31–34].

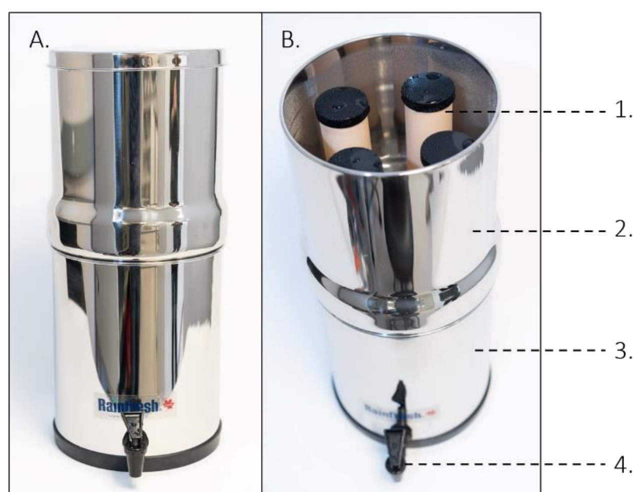

Figure 4.1: Filter setup from front (A) and top (B) views. 1. Four ceramic cartridges 2. Upper reservoir 3. Lower reservoir 4. Spigot.

4.3.2 Research gaps regarding CWF manufacturing and household use

Microbiological evaluation of CWFs consists of a process called "challenge testing", where the filters are operated using water spiked with virus, bacteria and/or protozoa (or their surrogates) under controlled laboratory conditions to determine the number of microorganisms reduced by filtration and calculate a $\log_{10}$ reduction value (LRV – i.e., the order of magnitude reduction of microorganisms by the filter)[18,19]. Efficacy achieved by a

device or method (i.e., the LRV) is used to assign a level of performance (i.e., highly protective, protective and interim) to contextualize the result and is the preferred method to both establish the microbiological protection conferred upon the end user[19] and ensure effective treatment of water by ceramic filtration[187]. There are two key research gaps associated with the current model of laboratory-based microbiological evaluation.

Firstly, in general CWFs must be sent from factories to centralised, offsite laboratories for microbiological evaluation, as microbiological testing is not always locally available due to lack of infrastructure, equipment and/or trained personnel[124], a common phenomenon in the low resource settings in which CWFs are used and manufactured (e.g., low- and middle-income countries). Due to these and other barriers, microbiological quality control (QC) testing is typically carried out on an average of 5% of all filters manufactured in low-resource contexts, and in some factories is not carried out at all[124]. Sufficient and accessible microbiological testing of CWFs remains a critical void, including reducing the cost, equipment and/or personnel requirements so that such testing can be more widely undertaken[124]. One possibility to help fill this void is a simplified field-based (i.e., without access to a laboratory) challenge test protocol to be implemented at the manufacturing site, to expand the accessibility of microbiological QC testing and more readily identify microbial risk-related filter weaknesses.

Secondly, although on-site microbiological QC testing is an important tool that could be used to improve filter quality at the factory, high LRVs observed under controlled challenge test conditions do not necessarily translate into high LRVs under conditions of real use[20]. This could be due to myriad reasons such as filters becoming cracked or damaged over time[24,25,100,101] with limited or no access to local supply chains to replace damaged parts[83,102]. Improper cleaning[25,82,83] and/or handling processes[21,82,84] also hinder CWF performance in the home compared to controlled challenge test conditions, as do general user practices such as dipping hands into filter receptacle[21] or using untreated water to rinse the drinking cup[83].

In application to either or both of these research gaps, field-based microbiological challenge testing has been envisaged[20] using spike organism(s) that are safe for human consumption (i.e., “food-safe”) and therefore can be used outside a laboratory setting, satisfying ethical and safety requirements. An appropriate bacterial surrogate (a probiotic health supplement containing non-pathogenic *E. coli*) was subject to preliminary validation through previous work[36] via an established surrogate selection framework[111]. The bacterial surrogate could

be used to develop a simplified field-based challenge test protocol to be implemented at the manufacturing site using established field microbial techniques[188]. This would aid in identifying microbial risk-related filter weaknesses that would otherwise go undetected based on current QC practices. Concurrently, the food-safe bacterial surrogate could be employed for household microbial challenge testing, where CWF users would be asked to treat a sample of water spiked with the food-safe surrogate (CWF users would not drink the spiked water). Trained enumerators would administer the field challenge test using field-appropriate water quality testing methods[188] to process influent and effluent water samples and calculate an *in situ* LRV[20].

4.3.3 Aim & objectives

The overall aim of this study was to aid in closing the two research gaps described above, by validating the previously-studied[36] food-safe probiotic *E. coli* against a standard laboratory strain of *E. coli* for use as a spike bacteria for microbiological testing of CWFs. This aim was divided up into two main objectives. The first objective was to determine the removal efficacy of CWFs with respect to the probiotic *E. coli*, compared the laboratory strain, over the simulated filter lifetime. The second study objective was to contextualize the removal efficacy findings from the work of the first objective, by measuring the morphological characteristics of particle size and zeta potential for the probiotic and laboratory *E. coli* strains.

4.4 Methods

4.4.1 Overview

Two strains were used in this study: probiotic *E. coli* (strain name Nissle 1917, or EcN, brand name Mutaflor®, Pharma-Zentrale GmbH, Herdecke, Germany), and a standard laboratory strain, *E. coli* K-12 (EcK12, ATCC 700926). To address the first objective described above (i.e., removal efficacy of CWFs with respect to EcN and EcK12), microbiological challenge testing was undertaken in the laboratory with non-silver-coated ceramic “candle” filters with an approximate 8 L capacity (Figure 4.1; Envirogard Products Ltd., Richmond Hill, Ontario, Canada). Three filters were evaluated in parallel with EcN or EcK12 as the spike organism (Figure 4.2). Each filter was assembled according to

manufacturer instructions, with one ceramic filter cartridge inside the top reservoir (Figure 4.1 shows four cartridges).

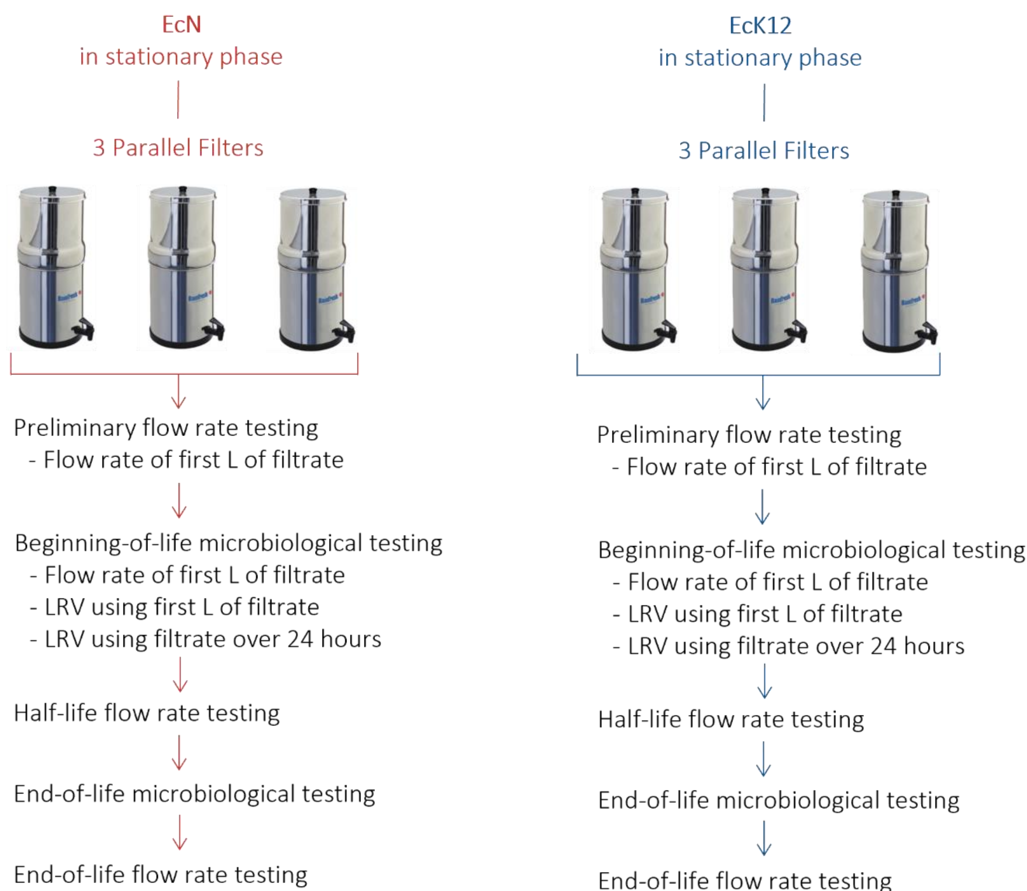

Figure 4.2: Overview of CWF challenge test procedure.

The second objective of the study was addressed by measuring zeta potential and particle size distribution (PSD) using a Malvern Zetasizer (Zetasizer Nano ZS90, Malvern Panalytical Ltd., Malvern, UK). Ceramic filters operate on three main mechanisms for particle and/or microorganism rejection: surface straining, which occurs when particles are physically retained at the filter surface because they are larger than the pores; adsorption, which occurs when particles small enough to enter the filter are adsorbed to the interior pore walls; and cake filtration, when particles that would be small enough to pass through the filter pores are retained by a cake of larger material that collects at the membrane surface[51]. That particle size plays a main role in physical retention at the filter surface is obvious, but particles smaller than the pore size can also be removed if the particles are retained on the interior pore surfaces via electrostatic attraction[189]. If the surface charge

of the particle and the filter media are both relatively low or oppositely charged, particles will tend to adhere to the interior pore surfaces, making it possible for the filter to remove particles smaller than its nominal pore size[51,189]. The zeta potential is a proxy measurement of particle surface charge; together with size, it is an important property that determines filter effectiveness for a given particle.

Under field challenge test applications as envisaged in Chapter 2, spiked challenge water would be prepared by dissolving the EcN capsule powder into the appropriate water volume, without pre-culturing to growth phase. This is due to the potential to inadvertently culture a pathogenic microorganism during the pre-culture phase – even if the seed microbe is food-safe and aseptic technique is employed[20]. Therefore, zeta potential and PSD were measured under three conditions (Figure 4.3): 1) EcN incubated to stationary phase; 2) EcN dissolved from powder capsule directly into test water; and 3) EcK12 incubated to stationary phase. The second condition is reflective of field conditions, allowing comparison to the first condition to ascertain impacts of pre-culturing and growth phase. The first and third conditions can also be compared to ascertain differences in *E. coli* strain. Under each condition, seven independent batches of sample were prepared, and each sample was measured five times by the Zetasizer, for a total of n = 35 measurements under each condition.

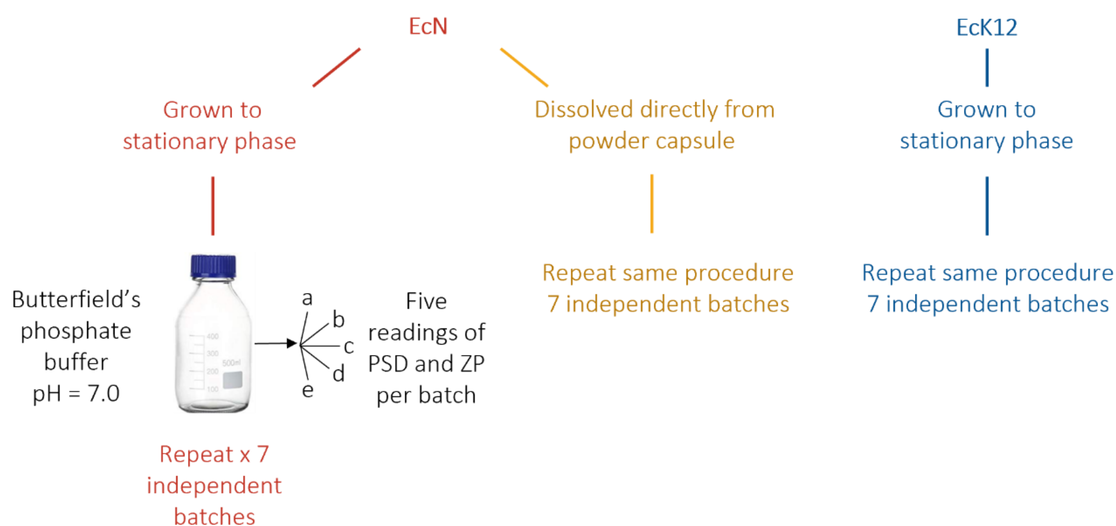

Figure 4.3:Overview of test procedure for zeta potential (ZP) and PSD

4.4.2 Bacteriological methods

EcN preparation methods

EcN dry powder capsules were kept refrigerated once received until use and tests were conducted before stated product expiry dates. For CWF challenge tests, EcN was incubated to stationary phase the day prior to testing[36] as follows: one capsule was externally sterilized by wiping the outside with 70% ethanol (Commercial Alcohols, Brampton, ON, Canada) and allowed to dry before aseptically opening and carefully depositing the powder contents into 100 mL of sterile Tryptic Soy Broth (TSB, Sigma Aldrich, St. Louis, Missouri, USA). The TSB/EcN mixture was incubated at 35°C overnight (16-18 hours) on a magnetic stirrer (500 rpm, lowest speed setting, Ika Topolino S1, IKA, Staufen, Germany).

For zeta potential/PSD tests, EcN was incubated to stationary phase as follows: the powder capsule was externally sterilized and aseptically transferred to approximately 100 mL of sterile buffered, quarter-strength isotonic Ringers solution (Oxoid Ltd., England, United Kingdom). The mixture was stirred on a magnetic stirrer (500 rpm) for approximately 5 minutes at room temperature to dissolve the powder. Once dissolved, a loopful of mixture was inoculated onto a nutrient agar slant (Tryptic Soy Agar, TSA, Sigma Aldrich, St. Louis, Missouri, USA) and incubated (Forced Air Microbiological Incubator 6.3CF, VWR, Radnor, Pennsylvania, USA) at 35°C overnight (16-18 hours) to reach stationary phase[36]. Cells were harvested by washing the agar surface twice with approximately 2 mL of Butterfield's phosphate buffer (BPB), then diluted to about 10 mL of Butterfield's phosphate buffer (BPB, described in Section 4.4.3). The mixture was stirred on a magnetic stirrer on lowest speed setting (500 rpm) for approximately 5 minutes at room temperature prior to zeta potential and PSD measurement.

For zeta potential and PSD measurements where EcN was dissolved directly from the powder capsule (i.e., no incubation step), EcN was dissolved into approximately 80 mL BPB directly prior to zeta potential and PSD measurement by aseptically transferring one capsule into BPB and stirring on low speed (500 rpm) for 5 minutes at room temperature.

EcK12 preparation methods

EcK12 was stored at -80 °C in Lysogeny broth (LB, Difco Laboratories, Franklin Lakes, New Jersey, USA) containing 50% (vol./vol.) glycerol and cultured onto TSA plates

(stored at 4°C) at approximately 3-month intervals. For CWF challenge tests, the day prior to testing, a loopful of culture was inoculated from the TSA plate to 100 mL of sterile TSB, and the mixture incubated overnight at 35°C while stirring (500 rpm). For PSD/zeta potential measurements, a loopful of culture was inoculated from the TSA plate to a TSA slant the day prior to testing, then incubated at 35°C overnight (16-18 hours) to reach stationary phase. After incubation, cells were harvested and transferred to 100 mL BPB as described for EcN.

Enumeration

For CWF challenge tests: if necessary, serial 10-fold dilutions were carried out using sterile buffered, quarter-strength isotonic Ringers solution. All samples were enumerated in triplicate using membrane filtration with Compact Dry™ EC plates (Nissui Pharmaceutical Co., Ltd., Japan). After 24 hours of incubation at 37°C[190], presumptive *E. coli* colonies were counted by visual inspection. If necessary (i.e., for test water before filtration, control samples), serial 10-fold dilutions were carried out prior to plating using sterile Ringers solution. For post-filtration runs, 100 mL of effluent water was plated. The lower limit of detection for plated samples was 1 CFU per volume of water plated.

4.4.3 Test water preparation

The CWFs were challenge tested using bottled mineral water from a groundwater source (Simply Pure, Victoria, British Columbia, Canada) as the matrix to avoid dechlorination as would be required if using tap water, and to simulate the typical water quality characteristics of groundwater. Water was prepared in 30 L batches for microbiological testing as follows: 1 L of mineral water was mixed with 15 mL of TSB containing either EcN or EcK12 at stationary phase, then briefly blended on low (1400 rpm, BBL620SIL Blender, Breville, Compton, CA, USA) to break up any bacteria clumps[19,191]. The mineral water-TSB mixture was then added to 29 L of mineral water (to make 30 L) and stirred manually with a paddle. The pH (pH probe IntelliCAL PHC101; multimeter MQ440D benchtop, both of the Hach Company, Loveland, Colorado, USA), UV absorbance at 254 nm wavelength (UVA_{254}, NanoPhotometer UV/Vis spectrophotometer, Implemen GmbH, Munich, Germany), and turbidity (2020WE turbidimeter, LaMotte, Chestertown, ML, USA) were measured prior to challenge testing. The test water was enumerated for *E. coli* prior to challenge testing (see Section 4.4.2 for enumeration methods).

Zeta potential and PSD measurements were conducted with Butterfield's phosphate buffer (BPB, adjusted to pH 7.0) as the medium[137]. First, a hydrogen-ion buffer was made by combining 290 mL and 500 mL of 0.1M solutions of NaOH and KH_2PO_4, respectively (both Fisher Chemical, Waltham, Massachusetts, United States), and diluting the mixture to make 1 L using type 1 ultrapure water (MilliQ Super-Q water purification system, Millipore Sigma, Burlington, MA, USA). BPB water was prepared by diluting 50 mL of hydrogen-ion buffer to 1 L with MilliQ water and adjusting the pH to 7.0 using 0.1 M H_2SO_4 (VWR Chemicals, Radnor, Pennsylvania, USA).

4.4.4 Microbiological challenge tests of CWFs

Flow rate testing was carried out on brand new filters using a non-spiked test water with the dual purpose of measuring initial filter flow rate and providing a baseline (i.e., negative control) for the flow rate test at the end of the filter life (described in Section 4.4.4; see Figure 4.2). The upper reservoir of each brand-new filter was filled with non-spiked mineral water (approximately 8 L). The flow rate was assessed by measuring the time to fill a sterile 1 L Nalgene bottle (Economy Wide-Mouth Plastic Bottle, HDPE, Cole-Parmer, Vernon Hills, Illinois, USA) placed within the lower reservoir underneath the ceramic cartridge outlet, to directly catch filter effluent (i.e., bypassing the lower filter reservoir). Once flow rate testing was completed, the remaining water was allowed to filter from the top reservoir into the bottom reservoir, with no further test water added, and the filter was allowed to run for 24 hours before commencing microbiological testing of the filtrate, during which time the cartridges did have time to dry out.

Microbiological challenge tests were conducted using methods adapted from established protocols[18,19]. Three CWFs were tested in parallel using either EcN or EcK12 (i.e., three filters were evaluated simultaneously using the same batch of spiked test water). Prior to microbiological testing, the filter was disinfected by wiping the inside surfaces of the upper and lower filter reservoirs with 70% ethyl alcohol and allowed to dry fully. Then, 1 L of alcohol was placed into the lower reservoir and allowed to run through the open spigot. Once dry, the filters were reassembled, and bacteriological testing commenced by filling each upper reservoir with approximately 8 L of test water containing either EcN or EcK12. A separate sterile 1 L Nalgene bottle was filled with spiked water and set aside in a dark cupboard at room temperature for the duration of the challenge test (i.e., 24 hours) as a die-off control.

The bacteriological removal of the filters was assessed for the first 1 L of filtrate and after 24 hours of filtration following the first 1 L sample. To capture the first 1 L of filtrate, a sterile 1 L Nalgene bottle (the outside wiped with alcohol directly prior) was placed inside the lower reservoir to directly catch the filtrate from the filter cartridge. The time to fill the 1 L bottle was recorded to calculate flow rate, and the filtrate was enumerated for *E. coli*. No further test water was added to the upper reservoir after this, and over the remaining 24 hours of testing, the filtrate was allowed to fill the lower reservoir. After 24 hours, the filtrate was sampled by opening the spigot and allowing the first 1 L to go to waste (flushing the spigot), then filling a sterile 1 L Nalgene with filtrate, which was subsequently enumerated for *E. coli*. At this time, the control sample was additionally enumerated.

Following the first bacterial challenge test, the filters were disassembled, and the cartridges allowed to air-dry, before cleaning them by scrubbing the ceramic material away using 120 grit sandpaper (Norton Abrasives, Worchester, MA, USA) according to manufacturer instructions[192]. The sandpaper scrubbing procedure is normally used for cleaning cartridges should they become clogged or dirty[192], however we scrubbed the cartridges to simulate repetitive cleaning. The cartridges were scrubbed until they reached a diameter indicating that they were halfway to the end-of-life diameter as specified by the manufacturer, measured by caliper (SERIES 500 Absolute Digimatic Caliper, Mitutoyo, Kawasaki, Japan). Then, the filters were reassembled, and a flow rate test was undertaken (Figure 4.2). Following the half-life flow rate test, the filters were once again disassembled, dried, and cleaned until they reached the end-of-life diameter as specified by the manufacturer. The filters were reassembled, and challenge testing repeated on filters at end-of-life. The diameter of the cartridges was 49.0 mm (95% CI 49.0; 49.1 mm), 47.3 mm (95% CI 47.1; 47.4 mm) and 45.8 mm (95% CI 45.6; 46.0 mm), at the beginning, half-life and end of filter life, respectively.

Directly following completion of microbiological challenge testing at the end of life, a non-spiked flow rate test was repeated on each filter, with the additional step of enumerating the filter effluent for *E. coli*. The final flush test had dual objectives to measure the end-of-life flow rate and ascertain the degree of retention of the bacterial spike within the filter matrix.

4.4.5 Measurement of zeta potential and PSD

Zeta potential and particle size distribution (PSD) were measured using BPB test water that was prepared with either EcN or EcK12 in stationary phase, or EcN dissolved from powder capsule directly into test water (Figure 4.3; see Section 4.4.2 for preparation methods). Then, for each independent batch of BPB test water (i.e., 7 batches for each condition), the Zetasizer was used to take a total of 5 measurements of zeta potential and PSD. The Zetasizer was equipped with a Helium-Neon laser (633 nm) source of light, with detection at a 90° scattering angle. Particle size is measured by the Zetasizer via Dynamic Light Scattering (DLS), an optical measurement technique that measures Brownian motion via laser and by analysing intensity fluctuations in the scattered light[193]. The relationship between the hydrodynamic particle size and its particle diffusion speed due to Brownian motion is used to correlate light intensity fluctuation to hydrodynamic particle size[193]. Thus, if the particle being measured is not spherical, as is the case for *E. coli*, the size obtained by Zetasizer will correspond to a sphere having the same average translational diffusion coefficient[193].

Zeta potential is measured by the Zetasizer via the particles' electrophoretic mobility (i.e., the movement of a particle towards an electrode of the opposite charge), which is a function of the particles' zeta potential and the applied voltage gradient, in a medium of given dielectric constant and viscosity. Electrophoretic mobility is measured via laser doppler velocimetry (LDV), an optical measurement technique whereby particle movement is detected via measurement of a fluctuating laser intensity signal (i.e., the rate of fluctuation is proportional to the speed of the particles, and therefore their zeta potential)[193].

4.4.6 Statistical Methods

Once collected, data were entered into Microsoft Excel and arithmetic means and 95% confidence intervals (CIs) were calculated for grouped data where applicable. Non-detect membrane filtration results were assumed to be one-half the limit of detection (i.e., if 100 mL of water was filtered for membrane filtration, and no *E. coli* colonies were detected, the assumed concentration was 0.5 CFU/100 mL).

Where statistical comparisons were made, the data were first checked against the normality assumption via the Shapiro-Wilk normality test; in cases where data had $n > 30$ (i.e., for PSD and zeta potential), we used parametric statistical analysis procedures under the

central limit theorem that sampling distributions tend towards normality for large sample sizes[194]. Data points for PSD and zeta potential were removed based on manufacturer-recommended quality criteria as in Table 4.1, and experiments were not repeated to compensate for removed data points as only a nominal number of data points were removed (see Section 4.5.2).

Normally distributed data were evaluated for statistical differences using parametric two-tailed T-testing for two groups or Analysis of Variance (ANOVA) for multi-group comparisons; the post-hoc Tukey Test was used to evaluate multiple pairwise differences. In the case of size distribution and zeta potential, comparisons between the three experimental conditions (i.e., EcK12 grown to stationary phase, EcN grown to stationary phase, and EcN dissolved from the capsule) were statistically blocked by batch (i.e., seven batches per condition, Figure 4.3). No adjustment was made for naturally-occurring E. coli die-off over the 24-hour filtration period because initial test water concentrations and their respective 24-hour control samples were not significantly different (see Section 4.5.1).

Non-normally distributed data were evaluated using non-parametric Wilcoxon signed-rank tests between two groups or the Kruskal-Wallis tests for multi-group comparisons; the post-hoc Dunn's Test was used to evaluate multiple pairwise differences. Correlations between LRV, filter flow rates, and cumulative volume filtered were checked by pairwise linear regression; R^2, Pearson's rho ρ, test statistics and p-values were computed for each comparison.

All statistical testing was conducted in R (version 4.2.1) using Rstudio (version 2022.07.2, build 576), with the Tidyverse (version 1.3.2), rstatix (version 0.7.0) and readxl (version 1.4.1) libraries. All statistical testing was considered significant at the $p = 0.05$ confidence level.

Table 4.1: Quality criteria used to evaluate PSD and zeta potential data.

Parameter	Quality criteria	Exclusion criteria	Description
Particle size distribution	Polydispersity Index (PdI)	> 0.5	Indicates the size distribution of the individual sample reading. Values greater than 0.7 indicate that the sample has a very broad size distribution is likely to be unsuitable for the DLS technique[195]; data with PdI over 0.5 is too polydispersed for the PSD value to be deemed reliable[195].
	Intercept of the correlation function	< 0.1 or > 1.0	The correlation function is used to relate laser light scattering (and therefore Brownian motion) to particle size. The intercept represents the signal to noise ratio and is a key quality criteria; recommended to be between 0.1 and 1.0[193,196].
	Cumulants fit error of the correlation function	> 0.005	Measures the fit error of the correlation function[193,196].
	In-range value	$< 90\%$	Indicates the overall quality of a given size distribution reading by looking at the difference between the measured baseline of the correlation function and the theoretical baseline[197]
Zeta potential	Data quality factor	> 1.0	Measures the signal to noise ratio of the fluctuating laser signal used in LDV[193,198].

4.5 Results

4.5.1 Ceramic filter challenge tests

LRVs

There was no statistical difference between the filter LRVs using EcN as a challenge organism compared to using EcK12 ($p = 0.06$; Figure 4.4). Kruskal-Wallis testing yielded no statistical differences between the LRV of any of the four test stage/microbe combinations (i.e., EcN with new filters and end of life, EcK12 with new filters and end of

life; $p = 0.16$). The average influent test water concentration was $2.29 \cdot 10^7$ CFU/100 mL (95% CI $1.88 \cdot 10^7$; $2.69 \cdot 10^7$ CFU/100 mL) and $4.28 \cdot 10^7$ CFU/100 mL (95% CI $4.17 \cdot 10^7$; $4.38 \cdot 10^7$ CFU/100 mL) for EcK12 and EcN, respectively. Raw data for ceramic filtration experiments is available in the SI Table S4.3. No significant differences were found between the initial test water concentrations and their respective 24-hour control samples ($p = 0.80$), and no significant die-off occurred in the 24-hour control samples ($p = 0.50$).

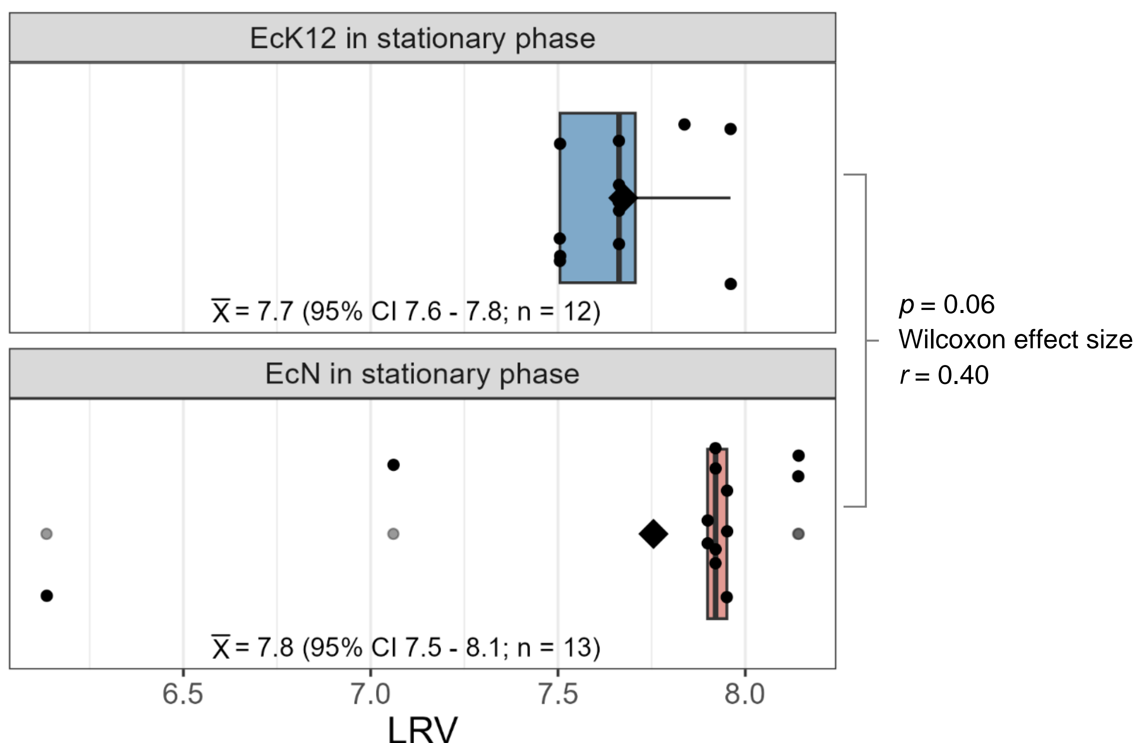

Figure 4.4: Results of CWF challenge testing. Box edges correspond to the upper and lower quartiles; the box therefore covers the interquartile interval (where 50% of the data is found). The vertical line splitting the box is the median; the diamond symbol is the sample mean. Comparisons on the right depict p*-values and mean differences (MD).*

There was no statistical difference in the filter LRVs at the beginning of the filter life compared to the end ($p = 0.19$), or the LRV seen with the first L of water filtered compared to the remaining water filtered over the subsequent 24-hour period ($p = 0.49$). The mineral water used in this study followed the "high-quality" category set by the WHO[19], with a mean turbidity of 0.4 NTU (95% CI 0.2; 0.5 NTU, n = 15), mean UVA_{254} of 0.005 (95% CI 0.001; 0.008, n = 15), and a pH of 7.1 (95% CI 7.1; 7.2, n = 15). Enumeration of control samples showed that spike bacteria in the test water did not show a significant decrease

in number over the 24-hour filtration time, with a mean decrease of 0.03 LRV (95% CI -0.1; 0.1 LRV).

Flow rate correlations

The overall mean flow rate of the filters in this study was 0.4 L/hour (95% CI 0.3; 0.5 L/hour; n = 34). There was no statistical difference in filter flow rate between the beginning and end of life ($p = 0.08$ using a two-sample non-parametric Wilcoxon rank sum test; data failed the normality assumption with $p = 0.01$ and $p = 0.08$ for the beginning and end of life, respectively). Table 4.2 shows a summary of correlate analysis between microbiological filter performance (i.e., LRVs), flow rate and cumulative throughput (i.e., the cumulative volume of water processed through the filter).

Table 4.2: Summary of simple linear model statistics applied to flow rate data.

Correlation	Pearson's ρ (95% CI)	R^2	p-value	Degrees of freedom
LRV as a function of cumulative throughput (L)	0.35 (-0.05; 0.66)	0.13	0.08	23
LRV as a function of flow rate (L/hour)	0.50 (-0.07; 0.82)	0.25	0.08	11
Flow rate (L/hour) as a function cumulative throughput (L)	0.25 (-0.10; 0.54)	0.06	0.16	32

4.5.2 PSD and zeta potential

Based on the manufacturer-recommended quality criteria[193,195,196,198], 3 of the original 105 data points (2.9%) for particle size were excluded from the final dataset for statistical analysis, while none of the original 105 data points (0%) for zeta potential were excluded.

With respect to particle size distribution (Figure 4.5), all three test conditions differed significantly from each other ($p < 0.01$ for all three pairwise comparisons); the mean difference (MD) in all cases was less than 0.2 µm (200 nm). For zeta potential (Figure 4.5), all three test conditions also differed significantly from each other ($p < 0.01$ for all three pairwise comparisons), with mean differences between 0.9 and 3.1 mV.

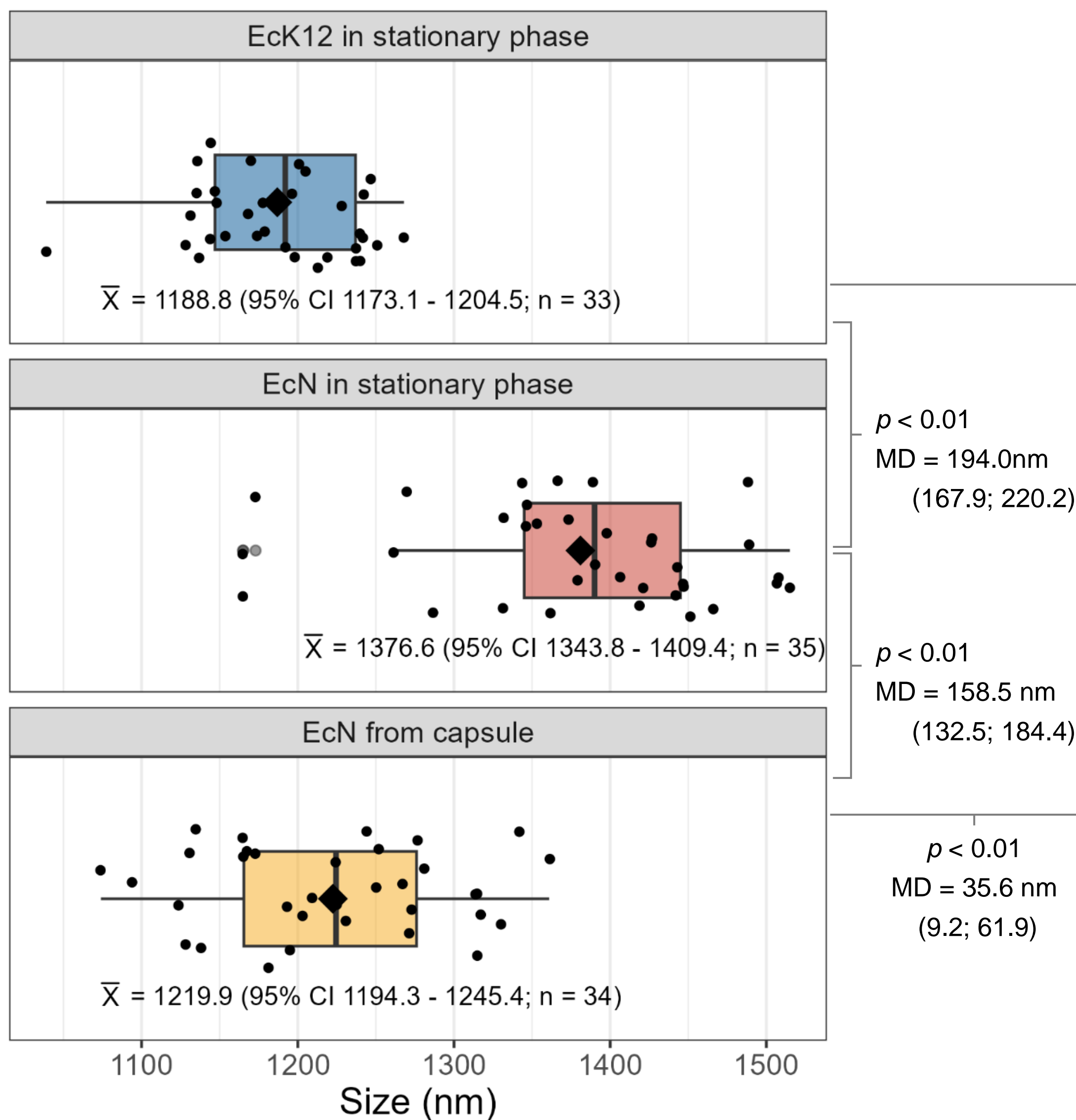

Figure 4.5: Particle size distribution (PSD) results, for EcN in stationary phase and dissolved directly from capsule, and EcK12 in stationary phase. Box edges correspond to the upper and lower quartiles; the box therefore covers the interquartile interval (where 50% of the data is found). The vertical line splitting the box is the median; the diamond symbol is the sample mean. Comparisons on the right depict p-values *and mean differences (MD).*

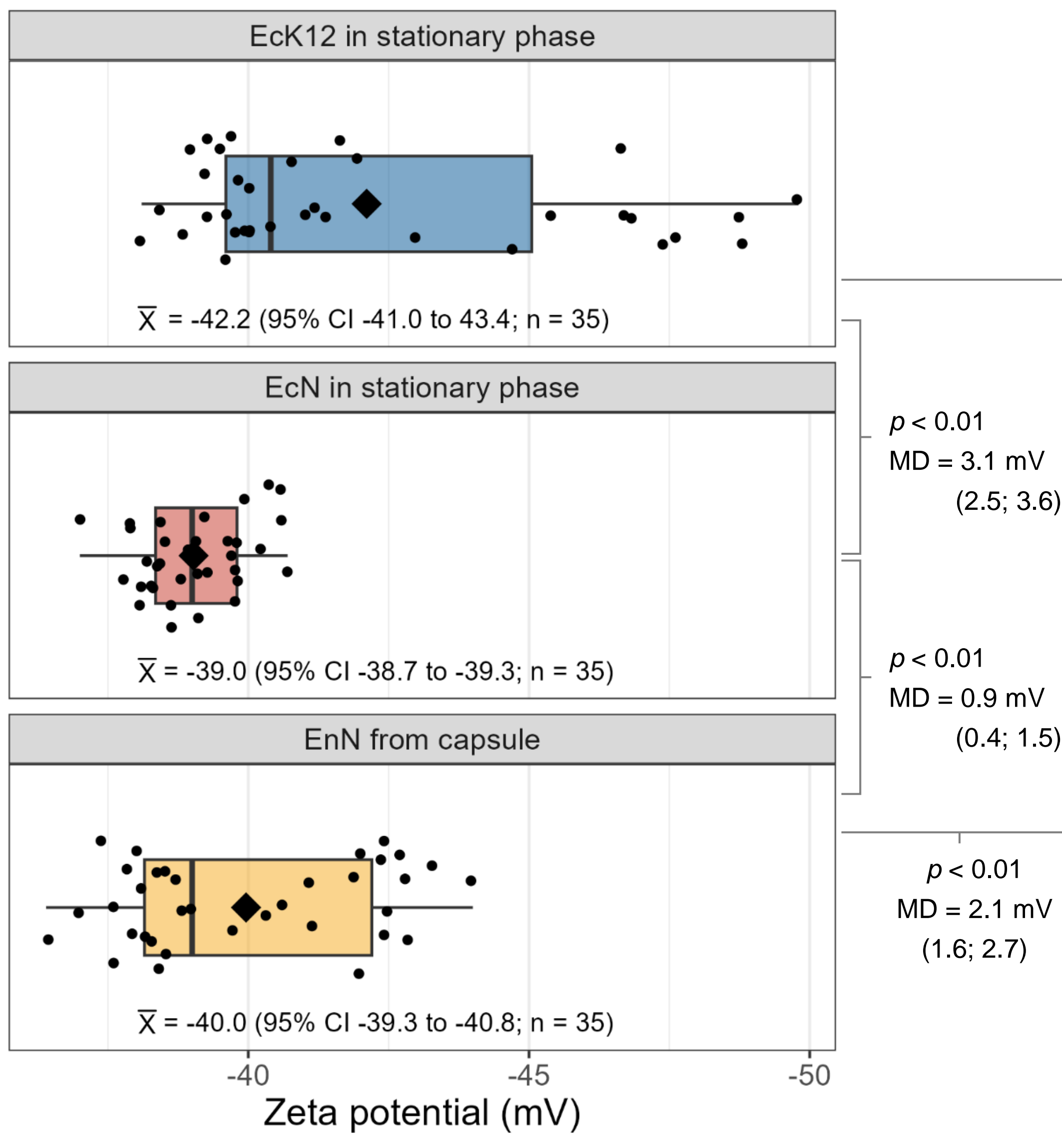

Figure 4.6: Zeta potential results, for EcN in stationary phase and dissolved directly from capsule, and EcK12 in stationary phase Box edges correspond to the upper and lower quartiles; the box therefore covers the interquartile interval (where 50% of the data is found). The vertical line splitting the box is the median; the diamond symbol is the sample mean. Comparisons on the right depict p-values and mean differences (MD).

4.6 Discussion

4.6.1 Filtration

LRVs

The filters reached relatively high mean LRVs for both EcN (7.7; 95% CI 7.6; 7.8) and EcK12 (7.7; 95% CI 7.5; 8.1) test organisms, which is more than an order of magnitude greater than the maximum expected laboratory effectiveness of such porous ceramic filtration methods (6 LRV[19]). Other studies of CWFs (both pot and candle style) report a very wide range of LRVs from non-silver-coated filters, from less than 1.0 LRV, to more than 8.2 LRV[199]. The results of our study are towards the higher end of that range, although it is worth noting that there is evidence of positive correlation between influent concentration and removal[99], and that our influent concentrations (approximately 10^7 CFU/100 mL) is on the upper range of that typically seen in the literature.

Influent microbe concentrations are one of numerous factors that can affect observed laboratory performance of CWFs and thus limit comparisons between studies. Such factors include selection and preparation of challenge microbes, enumeration methods, water quality parameters (i.e., turbidity or dissolved solids concentration), operational parameters (i.e., intermittent vs continuous flow), and filter cleaning procedures[19]. Based on current performance evaluation standards by the WHO[19] for bacteria only, the ceramic filters in this study would be classified as "highly protective" (i.e., bacterial LRV ≥ 4), although we can't wholly classify this as we didn't evaluate virus or protozoan pathogen classes in the current study as would be required to make a full classification.

Regarding the study objective to compare CWF removal efficacy between EcN and EcK12, we can say based on the lack of statistical difference that both strains responded similarly to ceramic filtration ($p = 0.06$ for non-parametric Wilcoxon T-testing; $p = 0.16$ for Kruskal-Wallis multi-group comparisons by test condition). CWF removal efficacy remained unchanged over the filter lifetime ($p = 0.19$) and between the first L of filtrate and that produced over the subsequent 24 hours of filtration ($p = 0.49$), therefore we can say that results are comparable between all conditions tested. Other researchers have found CWF performance to be slightly higher at the beginning of life, then to remain relatively unchanged throughout the filter lifespan[200].

However, the majority (21/25, or 84%) of filter effluent data were left-censored (i.e., results below the limit of detection of 1 CFU/100 mL), leading to right-censored calculated LRVs (i.e., the "true" LRV is unknown but above the calculated LRV[201]). For example, if the influent spike water had a concentration of $1.0 \cdot 10^7$ CFU/100 mL and the effluent results were left-censored (i.e., non-detect, with an assumed value of half the detection limit for LRV calculations), then the calculated LRV would be $7.3\log_{10}$ but the "true" value could be higher. If the influent spike had $2.0 \cdot 10^7$ CFU/100 mL with left-censored effluent, the right-censored calculated LRV would be 7.6. Therefore, the calculated LRVs in our results are effectively a function of the influent *E. coli* spike concentration, making it difficult to draw generalizations based solely on LRVs. To contextualize our right-censored LRV results, we look to the morphological characteristics of particle size and zeta potential in Sections 4.6.2 and 4.6.3.

Flow rate

Typical flow rates for ceramic water filters are between 1 – 3 L/hour[57,104,121]; ceramic filters produced with a flow rate outside of this range are sometimes discarded as a matter of quality control[57,82]. Flow rate is critically important to the ability of a household water treatment technology to ensure health gains; without sufficient quantity of water, the risk that the user will rely on additional untreated sources of water for drinking increases[121]. It is recommended that POUWT approaches be able to produce a minimum of 20 L per day[202]; some evaluation methods evaluate water production based on producing 20 L within 4 hours of applying the treatment (based on a 20 L per person per day with a 5-member household[121]).

The filters we studied had a mean flow rate of 0.4 L/hour (95% CI 0.3; 0.5 L/hour; n = 34), translating into 10.8 L/day, substantially lower than the minimum typical flow rates reported in the literature and half of the 20 L/day/person recommended by the WHO[202]. Filters made with more combustible material can result in a larger pore size and a lower microbial removal efficacy[58,203], and filters made with sand-rich clay can decrease the pore size and increase removal efficacy[204], although it is difficult to correlate LRV to flow rate within batches of filters (discussed below). Filters that maintain high LRVs and high flow rates are desirable for end users but remain a challenge, with calls to investigate into manufacturing parameters that can be modified to achieve this goal[54]. The filters in this study (Figure 4.1) were evaluated with only one ceramic filter cartridge installed but are intended to be run with four filter cartridges at a time, so theoretically the flow rate could

increase with the number of filter cartridges in use; however, it would likely not be a linear relationship (i.e., four times the filter cartridges does not mean four times the flow rate) as flow rate is also a function of pressure head which would remain unchanged with the installation of more cartridges.

Relationships between flow rate, LRVs, and cumulative throughput

We did not find any relationship between the filter LRV and the flow rate (Table 4.2; $p = 0.08$). Although flow rate is widely used as a quality control parameter[54,57,82,124,203] studies come down on both sides regarding correlation of flow rate to microbe reduction efficacy within filter batches, with some finding an association[200,205–207] and others not[36,58]. The LRV of our filters was not correlated to the cumulative throughput (Table 4.2; $p = 0.08$) and there was no statistical difference between LRVs at the beginning and end of the filter lifetime ($p = 0.19$), thus indicating that for the filters in this study, the LRVs remain consistent throughout the lifetime of the filters. Given that the main mechanism for filter bacterial removal efficacy is thought to be size exclusion via surface straining[54,199], it makes sense that $\log_{10}$ reduction would not decrease over the filter lifetime if cartridges are being cleaned at regular intervals according to manufacturer instructions.

Filter flow rate also was not correlated to the cumulative throughput (Table 4.2; $p = 0.16$), indicating that the flow rate remains consistent throughout the filters as studied. However, the filters were evaluated with relatively high-quality test water with a low turbidity of 0.4 NTU (95% CI 0.2; 0.5 NTU, n = 15); other studies show the tendency towards declining flow rate over filter lifetimes[121] and between filter cleaning, especially for high-turbidity water[24].

4.6.2 Bacteria size

E. coli is a typical gram-negative rod bacterium, usually 1000-2000 nm (1.0-2.0 µm) in length and with a radius of about 500 nm (0.5 µm)[208]. The mean hydrodynamic sizes of *E. coli* in this study are all within the documented typical range (i.e., 1188.8 nm, 1376.6 nm, and 1219.9 nm for EcK12 and EcN in stationary phase, and EcN Mutaflor from powder capsule, respectively; Figure 4.5).

The statistically significant difference in size between EcN and EcK12 grown on TSA ($p < 0.01$; Figure 4.5), and between EcN in stationary phase and from the powder capsule ($p < 0.01$; Figure 4.5) signifies that the two strains *of E. coli* are differently sized, and that

the growth conditions do have an impact on the bacteria size. This second finding is in agreement with literature that document the impacts of growth conditions (i.e., stage in growth curve, growth medium) on *E. coli* size[209,210]. Changes in *E. coli* mean length can be up to 1000 nm (1 µm) between lag and stationary phases of growth[209,210], and cell length at the point of division can vary by growth media by up to 1.5 times[211]. The mean size differences between EcN and EcK12 in stationary phase, and between EcN from the capsule were measured as 194.0 nm (95% CI 167.9; 220.2 nm) and 158.5 nm (95% CI 132.5; 184.4 nm), respectively (Figure 4.5). Cell size has been reported to vary by *E. coli* strain[212,213] by up to several micrometers in some cases[212]. The cell size distribution has also been noted to vary between *E. coli* strains, with some strains showing right-tailed distributions with high proportions of long cells[212].

Under use as a challenge organism in CWF field evaluations, EcN powder capsule would be directly dissolved into the challenge water, for reasons of logistical and safety constraint[20]. Under this use, field challenge test results would be compared to those observed in the laboratory, for example studies that use EcK12 as a challenge organism (grown to stationary phase). While the cell size of EcN dissolved from the powder capsule is statistically different to that of EcK12 in stationary phase ($p < 0.01$, Figure 4.5), the mean difference between the two is 35.6 nm (95% CI 9.2; 61.9 nm; Figure 4.5), or 0.0356 µm, the smallest mean difference of our three pairwise comparisons. For comparison, the average pore size of ceramic water filters has been reported as anywhere from 1.22 µm to 14.3 µm (i.e., 1220 nm to 14,300 nm)[54], varying depending on manufacturing factors such as firing temperature and/or ceramic composition[199]. Given the potential variation in porosity and pore size of ceramic filters, it seems likely that a mean size difference of 35.6 nm (0.0356 µm) will not affect the overall validity of EcN as a field-based filtration surrogate for EcK12.

4.6.3 Zeta potential

In a study of the zeta potential of EcK12 (DSM 498) in 0.75 mM NaCl solution, Schwegmann et al.[214] reported a zeta potential between -44.2 mV and -46.9 mV (no statistical information reported) at pH 7.0 to 7.2. Other studies report the zeta potential for other strains of *E. coli*: Halder et al.[215] reported a zeta potential of -44.2 mV (standard deviation of ± 0.50 mV) at pH 7.4 in 0.5 mM phosphate-buffered saline for *E. coli* MTCC 2939; Kłodzińska et al.[216] reported a zeta potential of -38.9 mV (no statistical information

reported) at pH 7.5 in 5 mM phosphate-buffered saline for *E. coli* PCM 2561. Novak et al.[217] did not report the strain of the *E. coli* they studied, however they found a zeta potential of -54.0 mV (standard error of ± 4.0 mV) at pH 7.0 in 30 mM NaCl.

The mean zeta potential values we report in the present study (i.e., -42.2 mV, -39.0 mV and -40.0 mV for EcK12 and EcN in stationary phase, and EcN from powder capsule, respectively) are comparable to the values found in the literature. All three pairwise comparisons (Figure 4.6) yielded statistical differences ($p < 0.01$ for all), signifying that EcN and EcK12 have different zeta potentials and that growth conditions do impact zeta potential for EcN. As mentioned above, the proposed use of EcN is as a field challenge microorganism (i.e., dissolved from the powder capsule into the challenge water), to be compared to laboratory-obtained challenge test results, for example those using EcK12 in stationary phase[36]. The two conditions (EcN powder capsule and EcK12 in stationary phase) differ by 2.1 mV (95% CI 1.6; 2.7 mV; Figure 4.6), which is a small difference compared to the breadth of the range of zeta potential values found in the literature under similar experimental conditions.

Studies comparing the zeta potential or electrophoretic mobility (based on which zeta potential is calculated) of non-pathogenic laboratory strains of *E. coli* to the pathogenic O157:H7 strains have determined statistically significant differences between the strains over a range of pH values[218,219]. All the observed zeta potential values in this study fall within a comparable range to those found in the literature under similar conditions[214–217,220]; this range is between -54.0 mV to -38.9 mV. Therefore, a difference of 2.1 mV seems insufficient to discard EcN as a sensible surrogate for other laboratory strains of *E. coli*, such as EcK12. It would seem that the mean difference in zeta potential would not impact the ultimate goal of using EcN, which is to establish the performance of filters within the broader category of bacterial reduction.

Additionally, because CWFs generally have a negatively charged ceramic surface, CWFs with and without silver coating generally exhibit low affinity with negatively-charged particles (such as *E. coli*), meaning that the main mechanism for filter efficacy would be size exclusion[54], although some studies have investigated the possibility to make positively charged ceramic filters for capture of small negatively charged particles such as viruses[54,221].

4.6.4 Statistical difference does not mean practical difference

The WHO microbiological performance testing guidelines[19] allow for flexibility in choice of spike microorganism, although *E. coli* B (ATCC 11303) is suggested. This flexibility should plausibly include the EcN strain (i.e., Mutaflor®). Myriad non-pathogenic strains of *E. coli* are used in the laboratory to challenge test ceramic and other types of filters, often without measuring strain zeta potential or microorganism size to verify validity[21,25,82,84,95,96,172,177,222].

Based on the criteria set by Sinclair et al.[111], our investigation into EcN was as a surrogate for EcK12 (i.e., Benchmarking III: "...documenting the range of surrogate behaviors with confidence limits and then placing the target pathogens in the appropriate range on a full spectrum."). *E. coli* is a process indicator for the bacteria pathogen class[19] and a faecal surrogate in general[127]. Following on the surrogate attribute prioritization scheme by Sinclair et al.[111], we consider practical attributes such as safety and ease of detection to be of key importance for surrogates used in field based studies, such as the suggested use of EcN in the current work. We observed that EcN responded similarly to filtration as EcK12 (Figure 4.4), thereby satisfying another priority surrogate attribute.

When contextualizing the finding that the functional morphology characteristics we observed (i.e., size and zeta potential) were statistically different for all three conditions studied, it is worth keeping in mind

Although the functional morphology characteristics we observed (i.e., size and zeta potential) were statistically different for all three conditions studied, a statistically significant p-value does not mean that the effect size is significant in magnitude[223]. For examination of a statistically significant but not significantly meaningful effect size, we used the framework of clinical medical research[223–225]. In clinical trials, a clinical difference (also referred to as substantive, scientific, meaningful, practical, actual, material and/or applied difference[225]) is one that makes a difference to the overall outcome of interest[224] (i.e., clinical practice or patient health in the case of clinical trials). In our case, the outcome of interest is the filter LRV in response to the choice of challenge microorganism, and more broadly, the potential health protection offered by the filter. Therefore, the calculated mean differences in morphology that we observed are not sufficient to significantly impact the response to filtration.

All surrogate selections involve decision trade-offs[111,226,227] as there is no such thing as a perfect surrogate, but as stated by Sinclair et al.[111], "the ultimate goal of selecting a sufficiently representative surrogate is to improve public health through a health-based risk assessment framework." Under this goal, we consider EcN to be imperfect but still representative of the bacterial reduction of CWFs, and preferable to the alternate, which would be continued barriers towards addressing the research gaps as identified in Chapter 2Section 4.3.2.

Although generally advantageous as described in Section 4.3, the open-source CWF production model lends itself to a wide field of input material (i.e., burnout and clay materials, including sieving and uniformity) and production methods (i.e., clay to burnout material ratio, mold construction; kilns and firing; silver application)[57,124,228,229]. Therefore, variation can and does occur between and within factories, with the potential outcome compromised performance (i.e., microbiological protection) of CWFs afforded to the end user[184,230–233]. To this end, filter manufacturers have been recommended to conduct repeated microbiological testing, especially in cases where a new CWF recipe is developed or if there is variation in input materials[58]. Not only would on-site techniques (i.e., using EcN to conduct challenge testing at manufacturing sites) decrease the burden of such microbial testing, but increased access to CWF testing could aid in improving quality control and production methods[124], including expanding current guidance documents and recommendations[57]. This would support filter manufacturers to produce, and therefore consumers to purchase, consistently high-quality technologies, while keeping knowledge and quality control practices local to the community[126].

Although the current work focuses on ceramic filtration, there is a wide range of HWTS technologies available, and an overarching knowledge gap regarding *in situ* evaluation of such technologies[112]. Use of field-appropriate surrogates such as EcN would garner more field-relevant data (i.e., under real-use conditions, where it matters most), thereby reflecting the influence of the contexts in which they are used, as opposed to idealized laboratory conditions[20].

4.6.5 Study limitations

The overall aim of the present study was to compare the behaviour of probiotic EcN with respect to filtration, in relation to a laboratory strain established for use in challenge test studies (EcK12). Therefore, the scope of performance testing of the CWFs was limited.

WHO protocols[19] recommend challenge testing CWFs using two types of test water: a high-quality test water with low turbidity, low organic content and relatively neutral pH; and a low-quality test water with higher turbidity, higher organic content and wider pH range. In the present study, we operated our CWFs using high-quality test water only, as the objective was to validate EcN as a surrogate, not to fully test the CWFs. It is likely that we would see increased filter clogging and lower flow rates if our CWFs had been studied under the low-quality water, as has been seen with other CWFs with high-turbidity water[24]. Although we simulated the filter lifetime by scrubbing the filter cartridge surface with sandpaper, we did not filter the large volumes of water (i.e., thousands of liters) as would be the case for CWFs over a typical lifetime of several years[54,181,234,235]. Because the focus of the current study was on validating the use of probiotic EcN to challenge test CWFs, we limited the study of the CWFs we used and did not examine the filter pore size or surface charge.

Examinations of CWFs as well as other membrane filtration methods (i.e., hollow fiber membrane filters) have found fouling, including biofouling, to be a barrier to continued CWF performance over the long term[179,236–238]. Fouling can be caused by particulates (e.g., turbidity), dissolved organic (e.g., natural organic matter, NOM) or inorganic matter (e.g., the precipitation of calcium carbonate, $CaCO_3$) accumulating within the filter pores[239]. Biofouling can also occur when microorganisms accumulate within filter pores to form a biofilm[101,239]. Biofouling in particular can cause cell-cell interactions during filtration that could have disparate impacts on the behaviour of EcN versus EcK12; this was not studied.

Ceramic filters have also been studied for non-biological contaminant removal, such as heavy metal removal[240]; this was not studied in the present work. It is common to produce CWFs impregnated with colloidal silver to potentially provide disinfection of viruses and prevent biofouling[27,57,178,199]. The filters used in our study did not contain silver, and it is therefore unclear what the effect of silver disinfection would be on EcN in comparison to EcK12, however we can make some inferences based on the known mechanisms of silver inactivation in CWFs. Silver disinfection is theorized to occur via two possible mechanisms: silver-bacterial interaction, which comprises bacteria-silver attachment and subsequent cell lysis; and reactive oxygen species (ROS) generation, in which silver species react with oxygen in the water to produce bactericidal ROS[199]. Electrostatic attraction (i.e., zeta potential) is thought to play a main role in the former disinfection mechanism, while the latter mechanism and associated kinetics has not been elucidated[199], although both gram-

positive and gram-negative bacteria have been shown to react with ROS, indicating that the electrostatic morphology may not have an outsize impact on ROS-based disinfection[241]. Silver disinfection efficacy in CWFs is greatly impacted by water quality characteristics, including pH, dissolved oxygen content (DO), and the presence of charged species and organic matter; silver characteristics, including quantity and chemical speciation of silver and any use of a chemical stabilization agent; and silver application methods, such as painting, co-firing and submerging methods[199]. Given the great variety of factors impacting silver disinfection, it seems unlikely that the choice between the two strains of *E. coli* we studied (with similar albeit statistically differing morphologies) would have an outsize impact on silver disinfection compared to the factors listed above.

Indeed, there are many strains of *E. coli* used as challenge organisms in the literature, and the WHO guidelines[19] allow flexibility on the choice of organism and strain without prior validation. The multitude of *E. coli* strains include those of environmental origin (i.e., wild-type)[82] or other non-K12 laboratory strains[84,96]. It is not feasible to validate probiotic EcN against all strains of *E. coli*, therefore we limited our investigation to EcK12, a common laboratory strain (e.g., [27,54,173]).

The evaluations in this study were conducted under controlled laboratory conditions that represent a "best case scenario" and the relatively high LRVs that were achieved in our study may not translate into high LRVs when CWFs are used in the home[20]. A key part of the health protection of POUWT devices is their adoption and sustained use[31–34], which is context-specific and therefore outside the realm of laboratory investigation.

Some studies examine the zeta potential of *E. coli* over a wide range of pH values[214,216,217,219] as this can greatly impact the zeta potential. However, as the objective of this work was to investigate the zeta potential of EcN for use in test waters mimicking drinking water, typically at a near-neutral pH, we limited our test conditions to pH 7.0 only.

4.7 Conclusion

There is a need for on-premises or easily accessible microbiological quality control for local CWF manufacturers to produce high-quality filters and therefore increase public health protection and consumer trust. The aim of this study was to validate the previously-studied probiotic *E. coli* (strain name Nissle 1917, or EcN, brand name Mutaflor®) against a standard laboratory strain of *E. coli* K12 (EcK12). The anticipated application of EcN

is for use as a challenge organism in on-site or decentralized microbiological quality control testing of CWFs, which would decrease some barriers towards more widely adopted microbiological quality control. Although we found statistically significant albeit modest morphological differences (i.e., size and zeta potential) between EcN and EcK12, both strains of *E. coli* responded similarly to ceramic filtration as studied under laboratory conditions. Based on our laboratory findings and additional priority surrogate attributes of safety and ease of field quantification, we conclude that EcN is a useful and feasible spike organism to represent the bacterial pathogen class in challenge testing.

4.8 Author contributions

Conceptualization: CZ and CCD; methodology: CZ, LPN and CCD; laboratory experiments: CZ, LPN and BC; statistical analysis: CZ and LPN; figure preparation: CZ; writing – original draft: CZ; writing – review and editing: CZ, LPN and CCD; supervision: CCD.

4.9 Supplementary information

Table S4.3: Raw data gathered from ceramic filtration experiments.

Preliminary Information / Cartridge Information

Test ID	Challenge (C_1L or C_24H), Flush (F) or Rate Test (R)	Challenge Bacteria: K-12 (K) or Mutaflor (M)	Time water added to top bucket	Approximate volume of water prepared, total (L)	Cartridge Number	Cartridge: new (N), half-life (H) or end-life (E)	Top Cartridge Diameter (mm)	Middle Cartridge Diameter (mm)	Bottom Cartridge Diameter (mm)	Average cartridge diameter (mm)
Mut_End_1L_Cart1	C_1L	M	2019-07-30 11:38	28	1	E	48.2	42.7	47.8	46.2
Mut_End_1L_Cart3	C_1L	M	2019-08-14 10:37	9.5	1	E	47.4	42.7	46.8	45.6
Mut_New_1L_Cart1	C_1L	M	2019-07-18 11:25	28	1	N	49.0	49.0	49.0	49.0
Mut_End_24H_Cart1	C_24H	M	2019-07-30 11:38	28	1	E	48.2	42.7	47.8	46.2
Mut_End_24H_Cart3	C_24H	M	2019-08-14 10:37	9.5	1	E	47.4	42.7	46.8	45.6
Mut_New_24H_Cart1	C_24H	M	2019-07-18 11:25	28	1	N		49.0	49.0	49.0
Mut_End_Flush_Cart1	F		2019-07-31 15:00	28	1	E	48.2	42.7	47.8	46.2
Mut_New_Flush_Cart1	F		2019-07-19 14:30	28	1	N	49.0	49.0	49.0	49.0
Rate_End_Cart1	R		2019-07-24 11:22	28	1	E	48.2	42.7	47.8	46.2
Rate_New_Cart1	R		2019-07-16 14:10	28	1	N		49.0	49.0	49.0
Mut_End_1L_Cart2	C_1L	M	2019-07-30 11:38	28	2	E	48.2	42.7	48.8	46.6
Mut_New_1L_Cart2	C_1L	M	2019-07-18 11:25	28	2	N		49.0	49.0	49.0
Mut_End_24H_Cart2	C_24H	M	2019-07-30 11:38	28	2	E	48.2	42.7	48.8	46.6
Mut_New_24H_Cart2	C_24H	M	2019-07-18 11:25	28	2	N	49.0	49.0	49.0	49.0
Mut_End_Flush_Cart2	F		2019-07-31 15:00	28	2	E	48.2	42.7	48.8	46.6
Mut_New_Flush_Cart2	F		2019-07-19 14:30	28	2	N		49.0	49.0	49.0
Rate_End_Cart2	R		2019-07-24 11:25	28	2	E	48.2	42.7	48.8	46.6
Rate_New_Cart2	R		2019-07-16 14:22	28	2	N	49.0	49.0	49.0	49.0
Mut_End_1L_Cart3	C_1L	M	2019-07-30 11:38	28	3	E	47.4	42.7	46.8	45.6
Mut_New_1L_Cart3	C_1L	M	2019-07-18 11:25	28	3	N	49.0	49.0	49.0	49.0
Mut_End_24H_Cart3	C_24H	M	2019-07-30 11:38	28	3	E	47.4	42.7	46.8	45.6
Mut_New_24H_Cart3	C_24H	M	2019-07-18 11:25	28	3	N		49.0	49.0	49.0
Mut_End_Flush_Cart3	F		2019-07-31 15:00	28	3	E	47.4	42.7	46.8	45.6
Mut_New_Flush_Cart3	F		2019-07-19 14:30	28	3	N	49.0	49.0	49.0	49.0
Rate_End_Cart3	R		2019-07-24 11:27	28	3	E	47.4	42.7	46.8	45.6
Rate_New_Cart3	R		2019-07-16 14:35	28	3	N		49.0	49.0	49.0
K12_New_1L_Cart4	C_1L	K	2019-08-08 12:23	28	4	N	49.2	49.0	49.2	49.1
K12_New_24H_Cart4	C_24H	K	2019-08-08 12:23	28	4	N	49.2	49.0	49.2	49.1
K12_Flush_24H_Cart4	F		2019-08-09 16:21	28	4	N	49.2	49.0	49.2	49.1
Rate_New_Cart4	R		2019-08-07 14:28	28	4	N	49.2	49.0	49.2	49.1
K12_End_1L_Cart5	C_1L	K	2019-09-17 10:55	28	5	E	48.0	41.5	47.6	45.7
K12_Half_1L_Cart5	C_1L	K	2019-08-28 11:40	28	5	H	48.3	44.4	48.1	46.9
K12_New_1L_Cart5	C_1L	K	2019-08-08 12:23	28	5	N	48.9	48.7	48.8	48.8
K12_End_24H_Cart5	C_24H	K	2019-09-17 10:55	28	5	E	48.0	41.5	47.6	45.7
K12_Half_24H_Cart5	C_24H	K	2019-08-28 11:40	28	5	H	48.3	44.4	48.1	46.9
K12_New_24H_Cart5	C_24H	K	2019-08-08 12:23	28	5	N	48.9	48.7	48.8	48.8
K12_End_Flush_Cart5	F		2019-09-18 14:00	28	5	E	48.0	41.5	47.6	45.7
K12_Flush_24H_Cart5	F		2019-08-09 16:21	28	5	N	48.9	48.7	48.8	48.8
K12_Half_Flush_Cart5	F		2019-08-29 12:09	28	5	H	48.3	44.4	48.1	46.9
Rate_New_Cart5	R		2019-08-07 14:28	28	5	N	48.9	48.7	48.8	48.8
K12_End_1L_Cart6	C_1L	K	2019-09-17 10:55	28	6	E	46.6	42.2	47.9	45.6
K12_Half_1L_Cart6	C_1L	K	2019-08-28 11:40	28	6	H	48.4	45.6	48.5	47.5
K12_New_1L_Cart6	C_1L	K	2019-08-08 12:23	28	6	N	49.0	49.0	49.4	49.1
K12_End_24H_Cart6	C_24H	K	2019-09-17 10:55	28	6	E	46.6	42.2	47.9	45.6
K12_Half_24H_Cart6	C_24H	K	2019-08-28 11:40	28	6	H	48.4	45.6	48.5	47.5
K12_New_24H_Cart6	C_24H	K	2019-08-08 12:23	28	6	N	49.0	49.0	49.4	49.1
K12_End_Flush_Cart6	F		2019-09-18 14:00	28	6	E	46.6	42.2	47.9	45.6
K12_Flush_24H_Cart6	F		2019-08-09 16:21	28	6	N	49.0	49.0	49.4	49.1
K12_Half_Flush_Cart6	F		2019-08-29 12:09	28	6	H	48.4	45.6	48.5	47.5
Rate_New_Cart6	R		2019-08-07 14:28	28	6	N	49.0	49.0	49.4	49.1
K12_End_1L_Cart10	C_1L	K	2019-09-17 10:55	28	10	E	46.0	41.2	47.4	44.9
K12_Half_1L_Cart10	C_1L	K	2019-08-28 11:40	28	10	H	48.4	45.6	48.2	47.4
K12_New_1L_Cart10	C_1L	K	2019-08-22 11:15	9.5	10	N	48.8	48.7	49.8	49.1
K12_End_24H_Cart10	C_24H	K	2019-09-17 10:55	28	10	E	46.0	41.2	47.4	44.9
K12_Half_24H_Cart10	C_24H	K	2019-08-28 11:40	28	10	H	48.4	45.6	48.2	47.4
K12_New_24H_Cart10	C_24H	K	2019-08-22 11:15	9.5	10	N	48.8	48.7	49.8	49.1
K12_End_Flush_Cart10	F		2019-09-18 14:00	28	10	E	46.0	41.2	47.4	44.9
K12_Half_Flush_Cart10	F		2019-08-29 12:09	28	10	H	48.4	45.6	48.2	47.4
Rate_New_Cart10	F		2019-08-23 12:27	9.5	10	N	48.8	48.7	49.8	49.1
Rate_New_Cart10	R		2019-08-21 13:58	9.5	10	N	48.8	48.7	49.8	49.1

Influent Information

Test ID	Time Influent Sampled	Influent Count A (CFU)	Influent Volume Filtered A (mL)	Influent Count B (CFU)	Influent Volume Filtered B (mL)	Influent Count C (CFU)	Influent Volume Filtered C (mL)	Calculated Influent concentration A (CFU/100 mL)	Calculated Influent concentration B (CFU/100 mL)	Calculated Influent concentration C (CFU/100 mL)
Mut_End_1L_Cart1	2019-07-30 12:00	48	0.0001	45	0.0001	41	0.0001	48000000	45000000	41000000
Mut_End_1L_Cart3	2019-08-14 11:00	46	0.0001	36	0.0001	37	0.0001	46000000	36000000	37000000
Mut_New_1L_Cart1	2019-07-18 11:25	48	0.0001	43	0.0001	34	0.0001	48000000	43000000	34000000
Mut_End_24H_Cart1	2019-07-30 12:00	48	0.0001	45	0.0001	41	0.0001	48000000	45000000	41000000
Mut_End_24H_Cart3	2019-08-14 11:00	46	0.0001	36	0.0001	37	0.0001	46000000	36000000	37000000
Mut_New_24H_Cart1	2019-07-18 11:25	48	0.0001	43	0.0001	34	0.0001	48000000	43000000	34000000
Mut_End_Flush_Cart1										
Mut_New_Flush_Cart1										
Rate_End_Cart1										
Rate_New_Cart1										
Mut_End_1L_Cart2	2019-07-30 12:00	48	0.0001	45	0.0001	41	0.0001	48000000	45000000	41000000
Mut_New_1L_Cart2	2019-07-18 11:25	48	0.0001	43	0.0001	34	0.0001	48000000	43000000	34000000
Mut_End_24H_Cart2	2019-07-30 12:00	48	0.0001	45	0.0001	41	0.0001	48000000	45000000	41000000
Mut_New_24H_Cart2	2019-07-18 11:25	48	0.0001	43	0.0001	34	0.0001	48000000	43000000	34000000
Mut_End_Flush_Cart2										
Mut_New_Flush_Cart2										
Rate_End_Cart2										
Rate_New_Cart2										
Mut_End_1L_Cart3	2019-07-30 12:00	48	0.0001	45	0.0001	41	0.0001	48000000	45000000	41000000
Mut_New_1L_Cart3	2019-07-18 11:25	48	0.0001	43	0.0001	34	0.0001	48000000	43000000	34000000
Mut_End_24H_Cart3	2019-07-30 12:00	48	0.0001	45	0.0001	41	0.0001	48000000	45000000	41000000
Mut_New_24H_Cart3	2019-07-18 11:25	48	0.0001	43	0.0001	34	0.0001	48000000	43000000	34000000
Mut_End_Flush_Cart3										
Mut_New_Flush_Cart3										
Rate_End_Cart3										
Rate_New_Cart3										
K12_New_1L_Cart4		16	0.0001	14	0.0001	18	0.0001	16000000	14000000	18000000
K12_New_24H_Cart4		16	0.0001	14	0.0001	18	0.0001	16000000	14000000	18000000
K12_Flush_24H_Cart4										
Rate_New_Cart4										
K12_End_1L_Cart5	2019-09-17 11:10	32	0.0001	15	0.0001	22	0.0001	32000000	15000000	22000000
K12_Half_1L_Cart5	2019-08-28 11:50	19	0.0001	21	0.0001	19	0.0001	19000000	21000000	19000000
K12_New_1L_Cart5		16	0.0001	14	0.0001	18	0.0001	16000000	14000000	18000000
K12_End_24H_Cart5	2019-09-17 11:10	32	0.0001	15	0.0001	22	0.0001	32000000	15000000	22000000
K12_Half_24H_Cart5	2019-08-28 11:50	19	0.0001	21	0.0001	19	0.0001	19000000	21000000	19000000
K12_New_24H_Cart5		16	0.0001	14	0.0001	18	0.0001	16000000	14000000	18000000
K12_End_Flush_Cart5										
K12_Flush_24H_Cart5										
K12_Half_Flush_Cart5										
Rate_New_Cart5										
K12_End_1L_Cart6	2019-09-17 11:10	32	0.0001	15	0.0001	22	0.0001	32000000	15000000	22000000
K12_Half_1L_Cart6	2019-08-28 11:50	19	0.0001	21	0.0001	19	0.0001	19000000	21000000	19000000
K12_New_1L_Cart6		16	0.0001	14	0.0001	18	0.0001	16000000	14000000	18000000
K12_End_24H_Cart6	2019-09-17 11:10	32	0.0001	15	0.0001	22	0.0001	32000000	15000000	22000000
K12_Half_24H_Cart6	2019-08-28 11:50	19	0.0001	21	0.0001	19	0.0001	19000000	21000000	19000000
K12_New_24H_Cart6		16	0.0001	14	0.0001	18	0.0001	16000000	14000000	18000000
K12_End_Flush_Cart6										
K12_Flush_24H_Cart6										
K12_Half_Flush_Cart6										
Rate_New_Cart6										
K12_End_1L_Cart10	2019-09-17 11:10	32	0.0001	15	0.0001	22	0.0001	32000000	15000000	22000000
K12_Half_1L_Cart10	2019-08-28 11:50	19	0.0001	21	0.0001	19	0.0001	19000000	21000000	19000000
K12_New_1L_Cart10	2019-08-22 11:30	37	0.0001	51	0.0001	49	0.0001	37000000	51000000	49000000
K12_End_24H_Cart10	2019-09-17 11:10	32	0.0001	15	0.0001	22	0.0001	32000000	15000000	22000000
K12_Half_24H_Cart10	2019-08-28 11:50	19	0.0001	21	0.0001	19	0.0001	19000000	21000000	19000000
K12_New_24H_Cart10	2019-08-22 11:30	37	0.0001	51	0.0001	49	0.0001	37000000	51000000	49000000
K12_End_Flush_Cart10										
K12_Half_Flush_Cart10										
Rate_New_Cart10										
Rate_New_Cart10										

Effluent Information

Test ID	Time Effluent Sampled	Total Effluent sampled	Effluent Count A (CFU)	Effluent Volume Filtered A (mL)	Effluent Count B (CFU)	Effluent Volume Filtered B (mL)	Effluent Count C (CFU)	Effluent Volume Filtered C (mL)	Calculated effluent concentration A (CFU/100 mL)	Calculated effluent concentration B (CFU/100 mL)	Calculated effluent concentration C (CFU/100 mL)
Mut_End_1L_Cart1	2019-07-30 13:09	1061.0	1	103.7	0	101.8	0	101.9	1.0	0.0	0.0
Mut_End_1L_Cart3	2019-08-14 13:00	839.0	0	99.5	0	102.8	0	101.1	0.0	0.0	0.0
Mut_New_1L_Cart1	2019-07-18 16:00	879.6	34	98.8	32	101.0	25	97.9	34.4	31.7	25.5
Mut_End_24H_Cart1	2019-07-31 11:30		0	100.5	1	103.3	0	124.6	0.0	1.0	0.0
Mut_End_24H_Cart3	2019-08-14 11:30		0	96.7	0	102.9	0	101.6	0.0	0.0	0.0
Mut_New_24H_Cart1	2019-07-19 11:15		0	98.8	0	101.0	0	97.9	0.0	0.0	0.0
Mut_End_Flush_Cart1	2019-07-31 17:00	801.0	0	101.2	0	105.1	0	101.9	0.0	0.0	0.0
Mut_New_Flush_Cart1	2019-07-19 17:47	1040.9	0	100.0	0	102.1	0	98.7	0.0	0.0	0.0
Rate_End_Cart1	2019-07-24 15:38	1410.3									
Rate_New_Cart1	2019-07-16 18:20	1026.8									
Mut_End_1L_Cart2	2019-07-30 13:09	1058.7	0	104.3	0	102.0	0	99.3	0.0	0.0	0.0
Mut_New_1L_Cart2	2019-07-18 16:00	961.5	0	98.8	0	95.6	0	100.0	0.0	0.0	0.0
Mut_End_24H_Cart2	2019-07-31 11:30		0	101.3	0	102.3	0	102.5	0.0	0.0	0.0
Mut_New_24H_Cart2	2019-07-19 11:15		0	98.8	0	95.6	0	102.3	0.0	0.0	0.0
Mut_End_Flush_Cart2	2019-07-31 17:00	730.1	2	106.6	0	105.0	0	108.2	1.9	0.0	0.0
Mut_New_Flush_Cart2	2019-07-19 17:47	1026.1	0	98.6	0	103.4	0	100.0	0.0	0.0	0.0
Rate_End_Cart2	2019-07-24 15:38	1319.4									
Rate_New_Cart2	2019-07-16 18:20	993.1									
Mut_End_1L_Cart3	2019-07-30 12:30	800.1	0	96.1	0	98.3	0	105.7	0.0	0.0	0.0
Mut_New_1L_Cart3	2019-07-18 16:00	957.6	1	100.0	3	100.8	7	101.5	1.0	3.0	6.9
Mut_End_24H_Cart3	2019-07-31 11:30		Contamination	102.9	Contamination	105.9	Contamination	103.1	Contamination	Contamination	Contamination
Mut_New_24H_Cart3	2019-07-19 11:15		0	100.0	0	100.8	0	91.5	0.0	0.0	0.0
Mut_End_Flush_Cart3	2019-07-31 17:00	911.3	0	103.4	0	99.6	0	105.0	0.0	0.0	0.0
Mut_New_Flush_Cart3	2019-07-19 17:47	1006.7	0	96.1	0	97.6	0	100.6	0.0	0.0	0.0
Rate_End_Cart3	2019-07-19 14:13	1122.9									
Rate_New_Cart3	2019-07-16 18:20	1192.2									
K12_New_1L_Cart4	2019-08-08 14:37	1418.7	101	98.8	115	105.1	83	103.9	102.3	109.4	79.9
K12_New_24H_Cart4	2019-08-09 12:32		42	99.3	30	102.3	36	102.4	42.3	29.3	35.2
K12_Flush_24H_Cart4	2019-08-09 18:00	928.5	47	101.2	63	104.6	59	103.1	46.5	60.2	57.2
Rate_New_Cart4	2019-08-07 17:18	1050.0									
K12_End_1L_Cart5	2019-09-17 14:41	923.5	0	96.1	0	95.8	0	99.8	0.0	0.0	0.0
K12_Half_1L_Cart5	2019-08-28 16:25	964.4	0	96.8	0	97.4	0	103.2	0.0	0.0	0.0
K12_New_1L_Cart5	2019-08-08 14:41	1203.0	0	103.7	0	105.3	0	101.4	0.0	0.0	0.0
K12_End_24H_Cart5	2019-09-18 11:10		0	94.9	0	97.4	0	101.7	0.0	0.0	0.0
K12_Half_24H_Cart5	2019-08-29 11:07		0	100.1	0	101.9	0	102.1	0.0	0.0	0.0
K12_New_24H_Cart5	2019-08-09 12:32		0	99.6	0	103.8	0	102.6	0.0	0.0	0.0
K12_End_Flush_Cart5	2019-09-18 15:33	893.4	3	100.6	4	98.6	3	104.2	3.0	4.1	2.9
K12_Flush_24H_Cart5	2019-08-09 18:00	964.1	0	99.1	0	104.3	0	99.9	0.0	0.0	0.0
K12_Half_Flush_Cart5	2019-08-29 14:00	916.9	0	97.8	0	99.3	0	101.0	0.0	0.0	0.0
Rate_New_Cart5	2019-08-07 18:46	655.7									
K12_End_1L_Cart6	2019-09-17 14:44	870.6	0	99.2	0	100.3	0	97.5	0.0	0.0	0.0
K12_Half_1L_Cart6	2019-08-28 16:27	917.6	0	99.0	0	99.6	0	102.1	0.0	0.0	0.0
K12_New_1L_Cart6	2019-08-08 14:46	1420.9	0	100.0	0	100.0	0	100.0	0.0	0.0	0.0
K12_End_24H_Cart6	2019-09-18 11:11		0	95.2	0	99.7	0	106.2	0.0	0.0	0.0
K12_Half_24H_Cart6	2019-08-29 11:12		0	100.0	0	98.1	0	106.7	0.0	0.0	0.0
K12_New_24H_Cart6	2019-08-09 12:32		0	96.4	0	103.9	0	100.4	0.0	0.0	0.0
K12_End_Flush_Cart6	2019-09-18 15:34	898.6	0	98.6	0	99.0	0	101.4	0.0	0.0	0.0
K12_Flush_24H_Cart6	2019-08-09 18:15	995.7	0	98.9	0	105.5	0	103.0	0.0	0.0	0.0
K12_Half_Flush_Cart6	2019-08-29 14:00	842.3	0	98.1	0	99.6	0	92.9	0.0	0.0	0.0
Rate_New_Cart6	2019-08-07 18:01	926.0									
K12_End_1L_Cart10	2019-09-17 14:46	1114.7	0	100.8	0	97.7	0	98.9	0.0	0.0	0.0
K12_Half_1L_Cart10	2019-08-28 16:33	1205.9	0	98.7	0	100.4	0	99.8	0.0	0.0	0.0
K12_New_1L_Cart10	2019-08-22 12:45	959.8	0	99.3	0	97.7	0	97.3	0.0	0.0	0.0
K12_End_24H_Cart10	2019-09-18 11:13		0	99.5	1	99.8	0	101.1	0.0	1.0	0.0
K12_Half_24H_Cart10	2019-08-29 11:15		0	100.9	0	103.6	0	97.0	0.0	0.0	0.0
K12_New_24H_Cart10	2019-08-23 11:30		0	96.5	0	93.8	0	100.0	0.0	0.0	0.0
K12_End_Flush_Cart10	2019-09-18 15:35	1209.9	0	107.1	0	97.6	0	104.8	0.0	0.0	0.0
K12_Half_Flush_Cart10	2019-08-29 14:00	835.8	0	103.5	0	95.8	0	103.1	0.0	0.0	0.0
Rate_New_Cart10	2019-08-23 14:07	952.0	0	102.5	0	99.7	0	98.3	0.0	0.0	0.0
Rate_New_Cart10	2019-08-21 17:31	1018.7									

Control Information

Test ID	Time Control Sampled	Control Count A (CFU)	Control Volume Filtered A (mL)	Control Count B (CFU)	Control Volume Filtered B (mL)	Control Count C (CFU)	Control Volume Filtered C (mL)	Calculated control concentration A (CFU/100 mL)	Calculated control concentration B (CFU/100 mL)	Calculated control concentration C (CFU/100 mL)
Mut_End_1L_Cart1										
Mut_End_1L_Cart3										
Mut_New_1L_Cart1										
Mut_End_24H_Cart1	2019-07-31 13:00	48	0.0001	34	0.0001	39	0.0001	48000000	34000000	39000000
Mut_End_24H_Cart3										
Mut_New_24H_Cart1	2019-07-19 16:30	38	0.0001	42	0.0001	28	0.0001	38000000	42000000	28000000
Mut_End_Flush_Cart1										
Mut_New_Flush_Cart1										
Rate_End_Cart1										
Rate_New_Cart1										
Mut_End_1L_Cart2										
Mut_New_1L_Cart2										
Mut_End_24H_Cart2	2019-07-31 13:00	48	0.0001	34	0.0001	39	0.0001	48000000	34000000	39000000
Mut_New_24H_Cart2	2019-07-19 16:30	38	0.0001	42	0.0001	28	0.0001	38000000	42000000	28000000
Mut_End_Flush_Cart2										
Mut_New_Flush_Cart2										
Rate_End_Cart2										
Rate_New_Cart2										
Mut_End_1L_Cart3										
Mut_New_1L_Cart3										
Mut_End_24H_Cart3	2019-07-31 13:00	48	0.0001	34	0.0001	39	0.0001	48000000	34000000	39000000
Mut_New_24H_Cart3	2019-07-19 16:30	38	0.0001	42	0.0001	28	0.0001	38000000	42000000	28000000
Mut_End_Flush_Cart3										
Mut_New_Flush_Cart3										
Rate_End_Cart3										
Rate_New_Cart3										
K12_New_1L_Cart4										
K12_New_24H_Cart4	2019-09-10 14:22	11	0.0001	16	0.0001	17	0.0001	11000000	16000000	17000000
K12_Flush_24H_Cart4										
Rate_New_Cart4										
K12_End_1L_Cart5										
K12_Half_1L_Cart5										
K12_New_1L_Cart5										
K12_End_24H_Cart5	2019-09-18 10:45	35	0.0001	40	0.0001	30	0.0001	35000000	40000000	30000000
K12_Half_24H_Cart5	2019-08-29 11:20	21	0.0001	29	0.0001	21	0.0001	21000000	29000000	21000000
K12_New_24H_Cart5	2019-09-10 14:22	11	0.0001	16	0.0001	17	0.0001	11000000	16000000	17000000
K12_End_Flush_Cart5										
K12_Flush_24H_Cart5										
K12_Half_Flush_Cart5										
Rate_New_Cart5										
K12_End_1L_Cart6										
K12_Half_1L_Cart6										
K12_New_1L_Cart6										
K12_End_24H_Cart6	2019-09-18 10:45	35	0.0001	40	0.0001	30	0.0001	35000000	40000000	30000000
K12_Half_24H_Cart6	2019-08-29 11:20	21	0.0001	29	0.0001	21	0.0001	21000000	29000000	21000000
K12_New_24H_Cart6	2019-09-10 14:22	11	0.0001	16	0.0001	17	0.0001	11000000	16000000	17000000
K12_End_Flush_Cart6										
K12_Flush_24H_Cart6										
K12_Half_Flush_Cart6										
Rate_New_Cart6										
K12_End_1L_Cart10										
K12_Half_1L_Cart10										
K12_New_1L_Cart10										
K12_End_24H_Cart10	2019-09-18 10:45	35	0.0001	40	0.0001	30	0.0001	35000000	40000000	30000000
K12_Half_24H_Cart10	2019-08-29 11:20	21	0.0001	29	0.0001	21	0.0001	21000000	29000000	21000000
K12_New_24H_Cart10	2019-08-23 11:30	14	0.0001	17	0.0001	10	0.0001	14000000	17000000	10000000
K12_End_Flush_Cart10										
K12_Half_Flush_Cart10										
Rate_New_Cart10										
Rate_New_Cart10										

Chapter 5: *Saccharomyces cerevisiae* as a protozoan surrogate: systematic review and meta-analysis of disinfection methods in drinking water treatment

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5.1 Abstract

Cryptosporidium parvum (*C. parvum*) and *Giardia lamblia* (*G. lamblia*) are key protozoan pathogens of concern in application to drinking water treatment (DWT). Where possible and appropriate, *C. parvum* and *G. lamblia* (oo)cysts should be used to quantify the performance of a given DWT technology, however there are various logistical and safety

challenges towards their use. Although there are numerous validated (non-)biological protozoan surrogates used to mitigate these challenges, they are typically limited in application to laboratory-scale validation testing and cannot support on-site or *in situ* testing of water treatment processes. As a field-appropriate alternative to current (non-) biological surrogates, food-safe *Saccharomyces cerevisiae* (*S. cerevisiae*, "baker's yeast") has been suggested as an (oo)cyst surrogate organism, however it has not been assessed in view of comparing the disinfection of *S. cerevisiae* to that of *C. parvum* or *G. lamblia* (oo)cysts. The overall objective of this systematic review was to evaluate *S. cerevisiae* as a protozoan surrogate in application to disinfection-based drinking water treatment methods.

We systematically reviewed three databases (PubMed, Web of Science and Engineering Village, published on or prior to August 18, 2020) for articles examining the dose-response behaviour of *S. cerevisiae* to UV, free chlorine, chlorine dioxide or ozone disinfection in water. We found 7887 records, of which 16 were retained for data extraction and meta-analysis following a review process by two blinded readers to prevent bias. Using a random-effects meta-analysis, we estimated that the UV disinfection dose required for $2\log_{10}$ reduction of *S. cerevisiae* is 203.1 J/m^2 (95% CI 133.2; 273.0 J/m^2). We therefore conclude that *S. cerevisiae* is a conservative surrogate for UV disinfection of *G. lamblia* (75.3 J/m^2; 95% CI 61.4; 89.2 J/m^2) and *C. parvum* (70.4 J/m^2; 95% CI 54.4; 86.5 J/m^2) at doses typically seen in drinking water disinfection. However, we were not able to draw any substantive conclusions regarding the suitability of *S. cerevisiae* as a surrogate for *G. lamblia* with respect to free chlorine, chlorine dioxide or ozone disinfection methods due to the paucity in available studies. To fully address the study objectives would require further laboratory investigation. This work was funded by the Natural Sciences and Engineering Research Council of Canada (NSERC).

5.2 Keywords

Drinking water disinfection; *Saccharomyces cerevisiae; Giardia lamblia; Cryptosporidium parvum.*

5.3 Introduction

5.3.1 Protozoan pathogens of concern in drinking water

Cryptosporidium parvum (*C. parvum*) and *Giardia lamblia* (*G. lamblia*) are protozoan parasites of concern in application to drinking water treatment (DWT)[242,243] and are one of the largest sources of health risk to municipal water systems in Canada[244]. The ubiquitous occurrence of such parasites in the environment, together with the resistance to chemical disinfection has made them a common target and reference pathogens for health-based risk assessments of DWT systems, known as Quantitative Microbial Risk Assessments (QMRA)[128].

Typically, municipal DWT systems are granted presumptive $\log_{10}$ reduction values (LRVs) for processes that comply with established guidelines by relevant environmental and/or public health agencies; for example, systems in the USA and Canada comply with the United States' Environmental Protection Agency (USEPA) Long Term 2 Enhanced Surface Water Treatment Rule, LT2ESWTR[242,245]. LRV credits are allocated[242,245] based on the calculated disinfection profile and benchmark demonstrated based on observed concentration-contact time (CT) or intensity-contact time (IT) values for chemical- and UV-based disinfection methods, respectively[246]. Where an operator can demonstrate that a drinking water treatment plant (DWTP), or a unit process within a DWTP, consistently achieves a *C. parvum* and/or *G. lamblia* treatment efficiency greater than the presumptive LRV specified in the LT2ESWTR[245], the system may be allowed to receive a higher treatment credit based on a site-specific demonstration of performance (DOP) study[245].

For small-scale DWT systems, such as modular systems, point-of-use or point-of-entry water treatment devices, the effectiveness of such technologies in removing or inactivating *C. parvum* and *G. lamblia* protozoans is typically validated via challenge testing. Water is spiked with protozoans of concern *C. parvum* and/or *G. lamblia* (or their surrogates) under controlled laboratory conditions[18,19,247,248] and used in simulated operating conditions. The reduction efficacy achieved by a device or method can be used to assign a level of performance (e.g., highly protective, protective and interim) and contextualize the result using a health-based quantitative microbial risk assessment (QMRA)[19,134].

5.3.2 Why might protozoan surrogates be used?

Where possible and appropriate, *C. parvum* and *G. lamblia* (oo)cysts should be used in DOPs and quantify the performance of a given DWT technology[19,245], however there are various logistical and safety challenges towards their use. Conventional technologies for detection and enumeration of *C. parvum* and *G. lamblia* (oo)cysts, outlined in the USEPA Method 1623 [249] typically consist of three steps: filtration of a large volume of water sample, typically 10 to 20 L; concentration (using a centrifuge) of the presumed (oo)cysts; and microscopic identification and enumeration of the (oo)cysts using epifluorescence or nucleic staining techniques[242,249,250]. Such methods require a large burden of laboratory equipment to perform and are time-consuming, labour-intensive and technically complex, requiring a high degree of training for personnel[251,252]; the end result of which is that enumeration of *C. parvum* and *G. lamblia* cannot be widely implemented in some contexts due to cost or other logistical constraints[252].

Compounding logistical constraints, technical shortfalls of conventional detection and enumeration methods for *C. parvum* and *G. lamblia*[249] have been documented, including specific factors such as low or highly variable recovery rates of (oo)cysts from water samples[249,251,253]. Microorganism losses have been recorded during each step of the conventional enumeration process[254], in addition to difficulty isolating and correctly identifying (oo)cysts from other particles and debris present in environmental water samples[251] and a high capacity for human subjective error at each method stage[251]. Such factors can produce variable enumeration data with high false positive and false negative rates and poor precision and accuracy[251], which are reported to account for a significant portion of variance in health risk estimates pertaining to drinking water[255].

High cost burden, technical difficulty and variable (oo)cyst recovery rates related to conventional detection and enumeration methods make it difficult to implement and enforce DWT testing protocols and health-based standards solely based on *C. parvum* and *G. lamblia* counts[250,251,256]. In addition, it is impractical from a cost, logistical and safety standpoint to procure, culture and utilize (oo)cysts for DWT performance validations (DOPs) and efficacy testing[19], in particular in low-resource contexts or for larger scale or online/operational systems.

Due to the reasons outlined above, use of a validated non-pathogenic surrogate for *C. parvum* and *G. lamblia* is an effective tool to evaluate DWT systems[18,19,245,256,257]. An ideal

surrogate should satisfy the following criteria: be present in the test waters in numbers that are directly related to the pathogen in question; not reproduce within the water matrix; be at least as resistant to the given treatment method to provide a conservative estimate of $\log_{10}$ reduction; and be feasible to enumerate using methods that are both specific and sensitive[258,259]. The search for surrogates of *C. parvum* and *G. lamblia* (oo)cysts is a challenge as they survive for long periods of time in the environment and *C. parvum*, in particular, is highly resistant to chemical disinfection[259].

5.3.3 Knowledge gap for biological surrogates

Although there are numerous validated (non-)biological protozoan surrogates (described in Section 5.4 below), their use is typically limited to laboratory-scale validation testing[260], or if used in large-scale or *in-situ* DWT systems, logistically challenging due to the ethical requirement that the water produced by such systems not be consumed, even if such surrogates are non-pathogenic. As an alternate to the surrogates reviewed in Section 5.4, food-safe *Saccharomyces cerevisiae* (*S. cerevisiae*, "baker's yeast") has been investigated as an (oo)cyst surrogate organism, and has been used to evaluate the performance of an existing stormwater recycling system[39] and a wastewater treatment/agricultural reuse system[261]. *S. cerevisiae* has been validated for use in filtration-based DWT technologies[37,38], was originally used in the NSF/ANSI Standard 55 for testing UV point-of-entry household water treatment systems[112], and has been recommended for further use in *in-situ* evaluations of stormwater and water reuse systems[41]. It has also been suggested that *S. cerevisiae* be used for field evaluations of point-of-use (POU) water treatment devices[20], for example free chlorine disinfection or solar disinfection, which are commonly used in low-resource contexts to treat water and protect against waterborne illness[4]. Current approaches to test PoU devices typically involve laboratory-based challenge testing, generating results that are not necessarily reflective of real life situations[19,20]; use of *S. cerevisiae* as a spike organism for field studies of PoU would allow incorporation of user conditions and source water quality into LRV estimates.

S. cerevisiae is safe for human consumption, in addition to being easy to obtain and transport to *in situ* evaluation sites. There are several established enumeration methods for *S. cerevisiae*, including spread or pour plate methods[262–264], the 3M Petrifilm method[265], and the SimPlate method[118,266] which is comprised of pre-packaged, pre-sterilized

components that do not need to be refrigerated, therefore making it more appropriate for *in situ* validation work or under-resourced settings[188].

S. cerevisiae is used in everyday food applications, such as breadmaking and brewing, and is established in food and beverage industry applications as a spoilage organism of interest[119,267], meaning that there exist numerous disinfection studies in various media, including water. Despite the potential for *S. cerevisiae* to be used as a consumer-safe surrogate in application to DWT, such disinfection studies have not been assessed in view of comparing the disinfection of *S. cerevisiae* to that of *C. parvum* or *G. lamblia* (oo)cysts. The present study aims to fill this gap with a systematic review and meta-analysis of water disinfection studies of *S. cerevisiae*.

5.3.4 Aim & objectives

The overall aim of this systematic review is to evaluate *S. cerevisiae* as a protozoan surrogate in application to disinfection-based drinking water treatment methods, and this can be divided into two objectives as follows:

1. Systematically examine the response of *S. cerevisiae* to typical disinfection techniques used in drinking water treatment; and
2. Compare such responses to established data by the USEPA and others for *C. parvum* and *G. lamblia*, protozoans of common concern to drinking water quality.

5.4 Background on other validated surrogates for *C. parvum* and *G. lamblia*

Numerous studies have been conducted to identify and validate surrogates for *C. parvum* and *G. lamblia* for use in evaluating conventional DWT methods (i.e., coagulation-filtration), biological treatment methods (i.e., slow sand filtration), and disinfection methods (i.e., UV or free chlorine). Such surrogates generally fall into two categories: biological and non-biological.

Non-biological surrogates for protozoan pathogens of concern (i.e., *C. parvum* and *G. lamblia*) include turbidity, particle counting and the use of (oo)cyst-sized (approximately 3-9 µm) microparticles[248]. Turbidity is used as a general measure of water quality, typically correlating with (oo)cyst abundance in surface waters[268,269]. A useful operational

parameter, known to correlate with parasite reduction during conventional DWT[270], particularly during filtration[257], turbidity is not a *C. parvum*- or *G. lamblia*-specific indicator[259] and cannot provide a direct measurement of treatment efficacy[271]. Similarly, microscopy-aided visual counts of (oo)cyst-sized particles have been reported to correlate with parasite reduction during conventional DWT[257,270,272]. However, such particle counts have been found to be significantly affected by water matrix factors such as turbidity, algae or other (non-oocyst) microorganism counts[270,273] and have therefore been recommended to only be used together with other monitoring tools such as turbidity and/or microbiological water quality testing[274].

Artificial microparticles, typically with diameter 3-9 µm, are commonly used as non-biological surrogates to conduct performance testing of DWT systems. They are chemically inert, easy to handle and relatively inexpensive[245], and have been validated for many specific DWT applications. Microspheres are made of various materials including glycoprotein- or biotin-coated microparticles[275,276], carboxylated latex spheres[277], polystyrene beads with or without fluorescent labelling[37,257,278,279], or monodispersed or co-modified polymer groups[280,281]. Such microspheres have been validated to assess protozoan pathogen reductions in evaluations of conventional DWT systems[277,280–282], biological treatment[282], filtration studies[257,275,277–279], UV disinfection[283–285], and ozone disinfection with[286,287] or without[288–290] free chlorine disinfection over a range of water matrices and operating conditions. Use of microspheres is endorsed by public health agencies to assess the performance of DWT systems for conventional DWT processes and filtration in particular agencies[19,248], however their use is limited to assessments in specific facilities and thus cannot provide on-site LRV determination or long-term monitoring for operation[260].

Biological surrogates for *C. parvum* and *G. lamblia* are also used for evaluating DWT methods. Spore-producing bacteria, typically aerobic *Bacillus subtilis* (*B. subtilis*), or anaerobic *Clostridium perfringens* (*C. perfringens*, also referred to as sulfite-reducing *Clostridia*)[19], have been validated as biological surrogates to evaluate the performance of traditional DWT techniques[245,258,291–293] and filtration methods[294–296], in particular those that function via adsorption or cationic surface attachment, such as carbon filters due to the similarity of surface charge to *C. parvum*[256]. Biological surrogates are more applicable than their inert non-biological counterparts to evaluate disinfection-based treatment methods[245], and have been validated for use with UV disinfection[297,298] and ozonation[299]. Aerobic spores in particular have elevated (although not as complete as *C. parvum*)

resistance to chlorination[300], and anaerobic spores have been used to validated chlorine-based disinfection systems[301], although both aerobic and anaerobic bacterial spores have been found to be inappropriate as direct indicators for *C. parvum* disinfection by chlorine dioxide[302]. Drawbacks for spore-related surrogates are that non-negligible laboratory resources are required to isolate, confirm, culture and propagate bacterial colonies, as well as create conditions for sporulation, creating barriers toward widespread adoption of performance testing using such surrogates[19]. As an alternate to sporulating bacteria, algae, in particular *Selenastrum capricornutum* and *Scenedesmus quadricauda,* have been used as surrogates for the removal of *C. parvum* by rapid sand filtration[303,304], although the use of algae is not widespread and some studies have found algae to be unsuitable for use as a surrogate for (oo)cysts[305].

5.5 Methods

5.5.1 Systematic Review

Overview

The systematic review was conducted following the guidelines of the Cochrane Handbook[306]. The checklists for Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) can be found in SI Figure S5.7 and Figure S5.8[307,308]. The study selection and review process were undertaken by two blinded readers (CZ and JW), with a third (CD) consulted in case of discrepancy, as a method to prevent bias. The review was not prospectively registered (e.g., with PROSPERO or INPLASY[309]).

Information sources

Three databases were searched: PubMed, Web of Science and Engineering Village. Studies published on or prior to August 18, 2020 were included for review. Only peer-reviewed, journal-published articles were reviewed, published in English, French, Spanish, Portuguese or Chinese.

Search strategy and quality control

The search strings used for the selected databases are presented in Table 5.1. Each section of the search string addressed one of four key concepts (target microorganism, disinfection method, synonyms for microorganism inactivation, water as the study matrix).

Table 5.1: Search strings used to search each selected database, with breakdown according to target concepts.

Database	Search String	Key Concept
PubMed & Engineering Village	(*saccharomyces* OR yeast OR *cerevisiae*) AND	Target microorganism
	(Chloramine* OR chlorin* OR boil* OR dioxide OR disinfect* OR hypochlor* OR irradiat* OR oxid* OR ozon* OR radiat* OR ultraviolet OR UV) AND	Disinfection method
	(apoptosis OR destruction OR "dieoff" OR "die-off" OR effective* OR inactivat* OR injur* OR mutat* OR reduction OR remov* OR survival OR treatment) AND	Inactivation synonyms
	(beverage OR flow OR fluid OR liquid OR solution OR water)	Water as study matrix
Web of Science	TS=(*saccharomyces* OR yeast OR *cerevisiae*) AND	Target microorganism
	TS=(Chloramine* OR chlorin* OR boil* OR dioxide OR disinfect* OR hypochlor* OR irradiat* OR oxid* OR ozon* OR radiat* OR ultraviolet OR UV) AND	Disinfection method
	TS=(apoptosis OR destruction OR "dieoff" OR "die-off" OR effective* OR inactivat* OR injur* OR mutat* OR reduction OR remov* OR survival OR treatment) AND	Inactivation synonyms
	TS=(beverage OR flow OR fluid OR liquid OR solution OR water)	Water as study matrix

Prior to undertaking the systematic search, nine articles[310–318] were identified for potential inclusion via a preliminary review. The nine articles were used to develop the search string (Table 5.1) using the listed article keywords, words in the title and abstracts, and keywords identified via a Yale MeSH (Medical Subject Headings) analysis[319]. The string was validated based on the inclusion of the nine articles in the search results.

Screening and study selection

A screening protocol was prepared in advance but was not prospectively registered (e.g., with PROSPERO or INPLASY[309]). In addition to study exclusion criteria based on publication type, language and date, inclusion and exclusion criteria were applied based on relevance to the research objectives.

Table 5.2: Exclusion criteria applied for study selection.

Key Concept	Inclusion Criteria	Exclusion Criteria
Target microorganism	Must examine *S. cerevisiae* (or yeast) as a test organism.	*S. cerevisiae* (or yeast) not examined.
Disinfection method	Methods must be applicable to common drinking water disinfection methods (e.g., UV disinfection). Must give details regarding dosage and contact time for given disinfection methods (e.g., J/m^2 or $mg \cdot min/mL$).	No disinfection method presented (e.g., yeast studied in relation to brewing or bioremediation). Disinfection method under study not applicable to drinking water treatment methods (e.g., ethanol disinfection, high-pressure CO_2 disinfection). Missing units or other key information related to calculation of disinfection dosage or contact time.
Inactivation	Must present inactivation data in response to the given disinfection method (e.g., graphical data, table of inactivation results).	Study solely the physiological recovery (e.g., dark repair) or DNA damage to *S. cerevisiae* in response to disinfection.
Water as study matrix	Must specify water as study matrix. Must use a standing sample of media (e.g., petri dish or cuvette).	Food, medical instruments or other solid surfaces as the study matrix. Study examines liquid flow (e.g., where liquid flows past a UV light with results as a function of flow rate).

To be included, the study had to examine a disinfection method relevant to drinking water treatment using methodology that emulates the conditions associated with drinking water treatment and is comparable to that used to generate dose-response estimates of *G. lamblia* and *C. parvum*. Inclusion and exclusion criteria were developed based on the framework of Stern et al.[320] (Table 5.2). Throughout this review, we use the widely-used term "dose" (with units of J/m^2) to refer to UV fluence, although "fluence" is the proper term[136]. This is for the sake of simplification, as "dose" is the proper term used in chemical disinfection studies (with units of $mg \cdot min/L$).

The systematic literature review proceeded in 4 stages using the reference management software Zotero (version 5.0.89) as follows:

1. Database search results imported into Zotero; duplicates removed.
2. Title and abstract screening (inclusion criteria listed in Table 5.2).
3. Full text screening and selection for study inclusion (criteria listed in Table 5.2).
4. Data extraction and quality assessment (detailed in next section).

5.5.2 Meta-Analysis

Data extraction

Once selected, data were extracted following the guidelines of Pedder et al.[321], by two blinded researchers (CZ and LPN), with a third (CD) consulted in case of discrepancy. The following data were extracted from each paper into a Microsoft Excel database: study authors and year; disinfectant type (i.e., UV, chlorine, ozone); water characteristics, pH, temperature; microbiological information such as strain name, growth phase (i.e., stationary or logarithmic), enumeration method and preparation steps; and statistical information as number of trials. UV-specific data such as lamp manufacturer information, wavelength of UV light, and dose intensity if specified (units of W/m^2) were recorded. If reported graphically, dosage (i.e., IT or CT values) with corresponding *S. cerevisiae* reductions were extracted via online WebPlotDigistiser (version 4.4)[322]; if results were tabulated in table format, they were extracted via Microsoft Excel. Depending how data were reported, Microsoft Excel was used to calculate $\log_{10}$ *S. cerevisiae* reductions, and IT or CT dosage was converted into a common set of units (i.e., J/m^2 for UV disinfection and $mg \cdot min/L$ for chemical disinfection methods).

Assessment of study quality and bias

Studies were rated for quality criteria summarized in Table 5.3 based on quality assessments by Bain et al.[323], Williams et al.[324], and Ramesh et al.[325], adapted from the CONSORT (consolidated standards of reporting trials[326]) and STROBE (strengthening the reporting of observational studies in epidemiology[327]) checklists. Since some criteria were relevant only to certain disinfection methods, the maximum number of points for each disinfection method differed and quality scores were normalized based on the maximum possible points (i.e., 17 points, 13 points, 13 points and 14 points for UV, free chlorine, chlorine dioxide and ozone disinfection, respectively). Studies achieving a quality score 69% or less were considered low quality; between 70 and 89%, medium quality; and 90% or above, high quality.

Table 5.3: Quality criteria used to assess studies.

Category	Index	Criterion	Question	Maximum points
Publication	1	Peer review	Was the study subject to peer review?	1
	2	Publication year	Is the publication published more recently than 1990?	1
Experimental design	3	Controls	Is a control sample used and the result reported? (i.e., dose = 0)	1
	4	Influent concentration	Is the pre-exposure microbe concentration reported? (Approximate values are OK)	1
	5	Water characteristics	Are water characteristics reported? (pH, water composition phosphate-buffered saline, temperature)	1
Microbiology	6	Strain information	Is the *S. cerevisiae* strain name given?	1
	7	Preparation methods	Are the microbiological preparation methods described? (e.g., incubation in nutrient broth to logarithmic phase)	1
	8	Enumeration method	Are appropriate enumeration methods described?	1
Statistics	9	Number of data points	How many data points do they report? 0 points if $\leq$ 2 data points 1 point if between 3 and 5 data points reported 2 points if 6 or more data points reported [a]	2

	10	Number of repeated tests	Are repetitions reported? (e.g., duplicate or triplicate testing)	1
	11	Statistical information	Is statistical information reported? (E.g., standard deviation or 95% CI on points)	1
UV specific	12	UV dose	Is the dose of UV light given for a given LRV, or can be reasonably calculated from the information provided?	1
	13	UV sensitivity of yeast	Is at least one non-UV-sensitive strain of yeast studied?	1
	14	UV wavelength	Is the wavelength of the UV light reported?	1
	15	Collimated beam characteristics	Are the petri factor and other spatial heterogeneity characteristics reported or described? [143,144]	1
	16	UV power	Is the power of the UV light reported? (i.e., W/m^2)	1
Chemical disinfection specific	17	Chemical dose	Is free chlorine, ozone or ClO_2 concentration reported? (i.e., dose can be calculated)	1
Ozone specific	18	Ozone bubbling rate	Is ozone bubbling rate reported?	1

[a] 6 points is the recommended minimum number by Bolton et al. [143,144] to construct an IT curve

Data analysis

A linear regression model was applied to each dose-response dataset following the Rennecker-Mariñas model[145] (Equation 5.1, adapted to base 10). N_0 and N are the number of microorganisms before and after the specified $Dose$ (i.e., IT or CT), k is the inactivation rate constant (also called the Chick-Watson coefficient of lethality per Chick[146] and Watson[147]), or the slope of the linear relationship, and b is the lag coefficient, or the dose below which the microorganisms do not exhibit significant inactivation[51].

Equation 5.1: The Rennecker-Mariñas model[145] for the dose-response of an organism to disinfection.

$$log_{10}\left(N/N_0\right) = \begin{Bmatrix} 0 \\ k_D \cdot (Dose - b) \end{Bmatrix} \begin{matrix} \text{for dose} < b \\ \text{for dose} \geq b \end{matrix}$$

For papers where dose-response data were reported for multiple conditions (e.g., logarithmic versus stationary phases of culture growth; multiple pH conditions), the linear regression model was applied to each condition separately. In cases where a long “tail” was

observed (i.e., inactivation activity decreasing at high dose values) data corresponding to the highest 5% of dose values were excluded from the linear model. In cases where a non-detect was reached, data for exposures past the first non-detect measurement were excluded. Once the dose-response models were obtained via linear regression, the disinfectant dose and respective 95% confidence intervals (CIs) required to achieve a $2\log_{10}$ reduction were extracted.

Due to the paucity of comparable dose-response data for the chemical disinfection methods, a meta-analysis was not possible and summary estimates have not been calculated. For UV disinfection, once the required dose (IT) for $2\log_{10}$ inactivation was calculated (i.e., the "effect estimate"), a random-effects meta-analysis was conducted to investigate summary and subgroup effects for each disinfection method were tabulated using the mixed-effects model[328]. Subgroup analysis included variables defined according to the test conditions in the studies, including logarithmic versus stationary phases and *S. cerevisiae* strain type. Meta-regression was used to investigate heterogeneity between UV disinfection studies, and to compare $2\log_{10}$ inactivation values to those reported by the USEPA for *C. parvum* and *G. lamblia*[329]. Potential publication bias was investigated via funnel plots[328].

Linear modelling and statistical analyses were conducted using Rstudio version 2022.07.2; additional open-source libraries were used: meta (version 6.1-0), readxl (version 1.4.1), tidyverse (version 1.3.2). Results from all statistical tests used in the analysis were considered significant at the $\alpha \leq 0.05$ significance level.

5.6 Results

5.6.1 Study selection

The initial literature search yielded 7887 results, of which 6005 individual records remained following the removal of duplicates (Figure 5.1). Following title, abstract and full-text screening for eligibility (Table 5.2), 22 articles[310–316,318,330–343] were identified for inclusion in the systematic review; following the quality assessment (Table 5.3), 16 articles[310–316,318,330,331,333,334,336–339] were identified for IT or CT dose-response data extraction and possible meta-analysis. During the quality assessment stage (see Table S5.8 for full quality assessment), the most common reason for exclusion was due to studies listing insufficient information with which to calculate dose-response data.

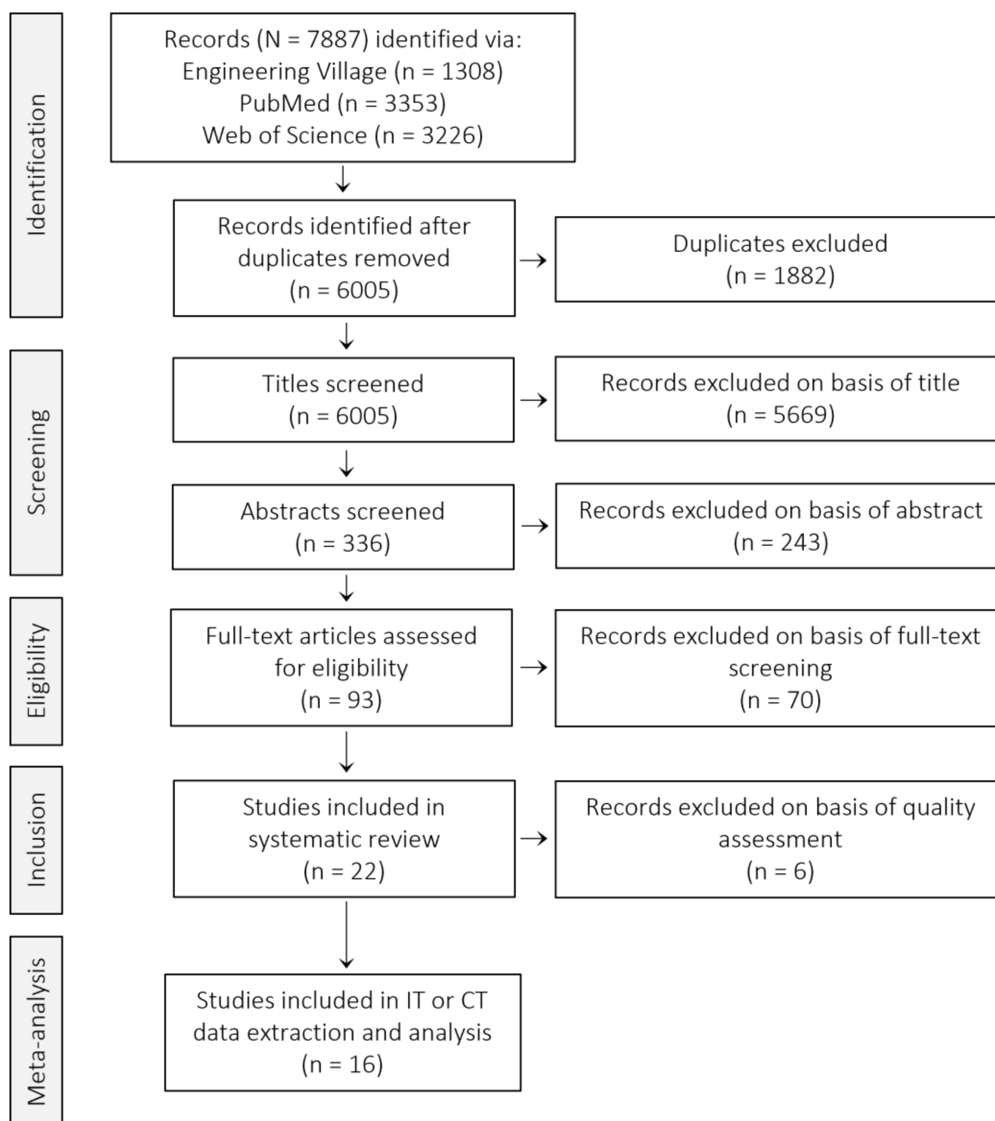


Figure 5.1: Results of database search and article screening.

5.6.2 Study characteristics

A summary of the selected studies produced by the systematic review and included for data extraction and analysis is presented in Table 5.4. Despite inclusion criteria for studies in English, French, Spanish, Portuguese or Chinese, all studies (22 studies, 100%) produced from the literature review were published in English.

Of the 16 included studies[310–316,318,330,331,333,334,336–339] for data extraction and analysis, UV was by far the most common method of disinfection studied (13 studies[310,312,315,316,318,330,331,333,334,336–339], or 81.2%). Of the 267 data points extracted from 16

studies, just under half the points (120 points, 44.9%) came from UV disinfection studies, while an almost equal number of points (111, 41.6%) came from two ozone disinfection studies[313,318]. There were very few overall data points regarding free chlorine or chlorine dioxide disinfection (36, 13.5%). Studies conducted testing with a variety of test water (distilled, mineral water, phosphate-buffered saline, acetate buffer; see Table 5.4), at a range of pH values, with a neutral (n = 6 or 37.5% of studies; n = 117 or 43.8% of data points) or mildly basic pH (pH 7.1-7.9, n = 6 or 37.5% of studies; n = 88 or 33.0% of data points) as the most common pH values. A full description of the characteristics and experimental conditions in each study can be found in Table S5.7.

Most studies excluded on the basis that they lacked information needed to calculate a dose-response relationship were assessed to be of low quality (n = 5 or 22.7% of studies[332,335,341–343]); no high-quality studies were excluded from the data extraction and analysis step. A surprising number of studies included for data extraction did not report statistical information such as standard error or confidence intervals on their data (n = 7 or 43.8% of studies[312–314,336–339]; n = 127 or 47.6% of data points), although nearly all reported conducting tests in at least duplicate (n = 15 or 93.7% of studies[310–313,315,316,318,330,331,333,334,336–339]; n = 259 or 97.0% of data points).

Table 5.4: Summary characteristics of studies produced from literature review and included for linear regression analysis. Note that some category percentages may add up to over 100% as some studies examined multiple factors.

Characteristic	Category	Number of studies produced from literature review	%	Number of studies included in linear regression	% (of included studies)	Number of data points included in linear regression	% (of included points)
Total		22	100.0	16	100.0	267	100.0
Disinfection method	Chlorine dioxide	1	4.5	1	6.3	16	6.0
	Free chlorine	2	9.1	2	12.5	20	7.5
	Ozone	2	9.1	2	12.5	111	41.6
	UV	19	86.4	13	81.3	120	44.9
Study quality	Low	9	40.9	4	25.0	23	8.6
	Medium	10	45.5	9	56.3	143	53.6
	High	3	13.6	3	18.8	101	37.8
Statistical information reported	Standard deviation	4	18.2	4	25.0	68	25.5
	Standard error	3	13.6	3	18.8	48	18.0
	95% CI	3	13.6	2	12.5	24	9.0
	Not specified	12	54.5	7	43.8	127	47.6
Number of reps	Duplicate	9	40.9	4	25.0	23	8.6
	Triplicate	11	50.0	10	62.5	191	71.5
	Quadruplicate	1	4.5	1	6.3	45	16.9
	Not specified	1	4.5	1	6.3	8	3.0
Language	English	22	100.0	16	100.0	267	100.0
	Other	0	0.0	0	0.0	0	0.0
UV wavelength, nm (UV papers only)	254	14	63.6	8	50.0	90	33.7
	266	1	4.5	1	6.3	6	2.2
	355	1	4.5	1	6.3	0	0.0

	Not specified	4	18.2	4	25.0	24	9.0
Strain type	Laboratory	8	36.4	4	25.0	66	24.7
	Wild-type	13	59.1	9	56.3	193	72.3
	Not specified	4	18.2	3	18.8	8	3.0
Growth phase	Logarithmic	9	40.9	6	37.5	57	21.3
	Stationary	14	63.6	12	75.0	210	78.7
	Not specified	1	4.5	0	0.0	0	0.0
Plating method	Pour plate	22	100.0	16	100.0	267	100.0
	Not specified	0	0.0	0	0.0	0	0.0
Water type	Acetate buffer	1	4.5	1	6.3	8	3.0
	Distilled	3	13.6	3	18.8	102	38.2
	Mineral	1	4.5	1	6.3	28	10.5
	PBS	8	36.4	8	50.0	99	37.1
	Peptone broth	1	4.5	1	6.3	4	1.5
	Saline	7	31.8	1	6.3	8	3.0
	YEPD broth [a]	1	4.5	1	6.3	14	5.2
	Not specified	1	4.5	1	6.3	4	1.5
pH	< 6.0	1	4.5	3	18.8	3	1.1
	6-6.9	1	4.5	1	6.3	42	15.7
	7.0	6	27.3	6	37.5	117	43.8
	7.1-7.9	6	27.3	6	37.5	88	33.0
	> 8.0	1	4.5	1	6.3	5	1.9
	Not specified	8	36.4	2	12.5	12	4.5

[a] *Yeast extract peptone dextrose broth.*

5.6.3 Meta-analysis

UV

The required UV dose to achieve $2\log_{10}$ reduction of *S. cerevisiae* in comparison to *C. parvum* and *G. lamblia* in water[329] is shown in Figure 5.2. The effect estimate (i.e., IT required for 2 LRV of *S. cerevisiae*) from Bisquert et al.[330] ($7.4 \cdot 10^5$ J/m^2) was excluded from the overall meta-analysis because it was several orders of magnitude higher than all other estimates (i.e., on the order of magnitude of $10^2 - 10^3$ J/m^2). The estimate came from only two data points quantifying dose-response, one of which was a control sample with no UV exposure. The UV dose for this single data point was relatively high ($2.5 \cdot 10^6$ J/m^2), somewhat explaining the very high dose-response value as calculated. The study also had a low quality score, 12 out of a possible 17 points (70.6%, low quality $< 71\%$).

The overall summary effect estimate for *S. cerevisiae* is 203.1 J/m^2 (95% CI 133.2; 273.0 J/m^2). There is a range of effect estimates yielding highly heterogenous results[328], with a pooled I^2 value of 99.8%. Subgroup analysis for reported IT values for baker's yeast showed a wide variety of IT values, resulting in a summary estimate of 251.9 J/m^2 (95% CI 3.7; 500.2 J/m^2). A summary of subgroup analyses is shown in Table 5.5; there was no statistical difference between the pooled subgroup estimates for strain types studied (laboratory modified vs wild-type vs baker's yeast) or the phase of culture growth (stationary vs. logarithmic).

Further analysis was conducted to investigate any further impacts of *S. cerevisiae* strain and/or growth phase (i.e., using *S. cerevisiae* grown to either logarithmic or stationary phase). Several laboratory strains were used in Sommer et al.[316] and the effect sizes (i.e., UV dose to achieve $2\log_{10}$ reduction of *S. cerevisiae*) have a high overall heterogeneity ($I^2 = 86.2\%$). However, baker's yeast and strains RC43a and YNN281 are not statistically different from one another and show minimal heterogeneity ($I^2 = 0.0\%$; $p = 0.55$). Strains YNN282 and YNN281xYNN282 are statistically different from baker's yeast and strains RC43a and YNN281 ($p < 0.01$ for both YNN282 and YNN281xYNN282), meaning that the effect size from these two strains drives the heterogeneity for the overall group of data from Sommer et al.[316].

Four papers[336–339] used the same *S. cerevisiae* strain (RAD 197/2d) in their UV dose-response investigations. Of these, three cultured *S. cerevisiae* to stationary phase[336,337,339];

these three effect estimates showed low to moderate heterogeneity and were not statistically different from each other ($I^2 = 39.8\%$, $p = 0.19$). Comparing stationary phase to logarithmic phase subgroups using meta-regression, we found that there is a statistical difference between the studies using *S. cerevisiae* in stationary phase and the study using *S. cerevisiae* in logarithmic phase[338] ($p < 0.01$).

Investigating the effect sizes from all wild-type *S. cerevisiae* strains, the overall wild-type subgroup had a high heterogeneity ($I^2 = 95\%$; Figure 5.2). Within the wild-type subgroup, studies conducted on strains under stationary and logarithmic phase conditions were also highly heterogenous ($I^2 = 85.3\%$ and 98.2%, respectively). However, meta-regression with wild-type *S. cerevisiae* showed no statistical difference between stationary and logarithmic phases ($p = 0.75$).

Three studies[310,312,316] looked at baker's yeast strains of *S. cerevisiae*. The two studies that examined baker's yeast in logarithmic phase[310,312] yielded effect sizes that statistically differed from each other ($p < 0.01$). But, comparing effect sizes from the two studies that examined baker's yeast in logarithmic phase[310,312] to the study that examined baker's yeast in stationary phase[316], we found no statistical difference ($p = 0.49$). Moustacchi and Enteric[333] was the only study that examined the same *S. cerevisiae* strain under both logarithmic and stationary phase conditions; the two conditions were statistically different ($I^2 = 96.3\%$, $p < 0.01$).

Table 5.5: Results from meta-regression to quantify subgroup heterogeneity for UV disinfection studies.

Variables	Number of studies (number of data points)	Estimate of UV dose required for 2 LRV, J/m^2 (95% CI)	between subgroups p-value
Strain type			
Baker's	3 (19)	252.4 (3.7, 501.0)	0.39
Laboratory modified [a]	1 (36)	118.4 (95.2, 141.7)	
Wild type	9 (63)	228.7 (127.0, 330.3)	
Growth phase			
Logarithmic [a]	5 (34)	283.8 (140.7, 427.0)	0.06
Stationary	8 (84)	125.5 (110.0, 141.0)	

[a] *All studies excluding Bisquert et al.*[330]

Meta-regression analysis is shown in Table 5.6; there was no statistical difference between the summary effects (i.e., the IT dose required for $2\log_{10}$ reduction) between *S. cerevisiae* and *C. parvum*, or *S. cerevisiae* and *G. lamblia*[329].

Table 5.6: Results of meta-regression comparing yeast IT values to protozoan pathogens.

Organism	Number of studies (number of data points)	Estimate of UV dose required for 2 LRV, J/m^2 (95% CI)	*p*-value in comparison with *C. parvum*	*p*-value in comparison with *G. lamblia*
Yeast [a]	13 (118)	203.1 (133.2, 273.0)	0.37	0.36
Baker's yeast only	3 (19)	252.4 (3.7, 501.0)	0.48	0.47
Cryptosporidium	1 (8)	75.3 (61.4, 89.2)	-	-
Giardia	1 (8)	70.4 (54.3, 86.5)	-	-

[a] *All studies excluding Bisquert et al.*[330]

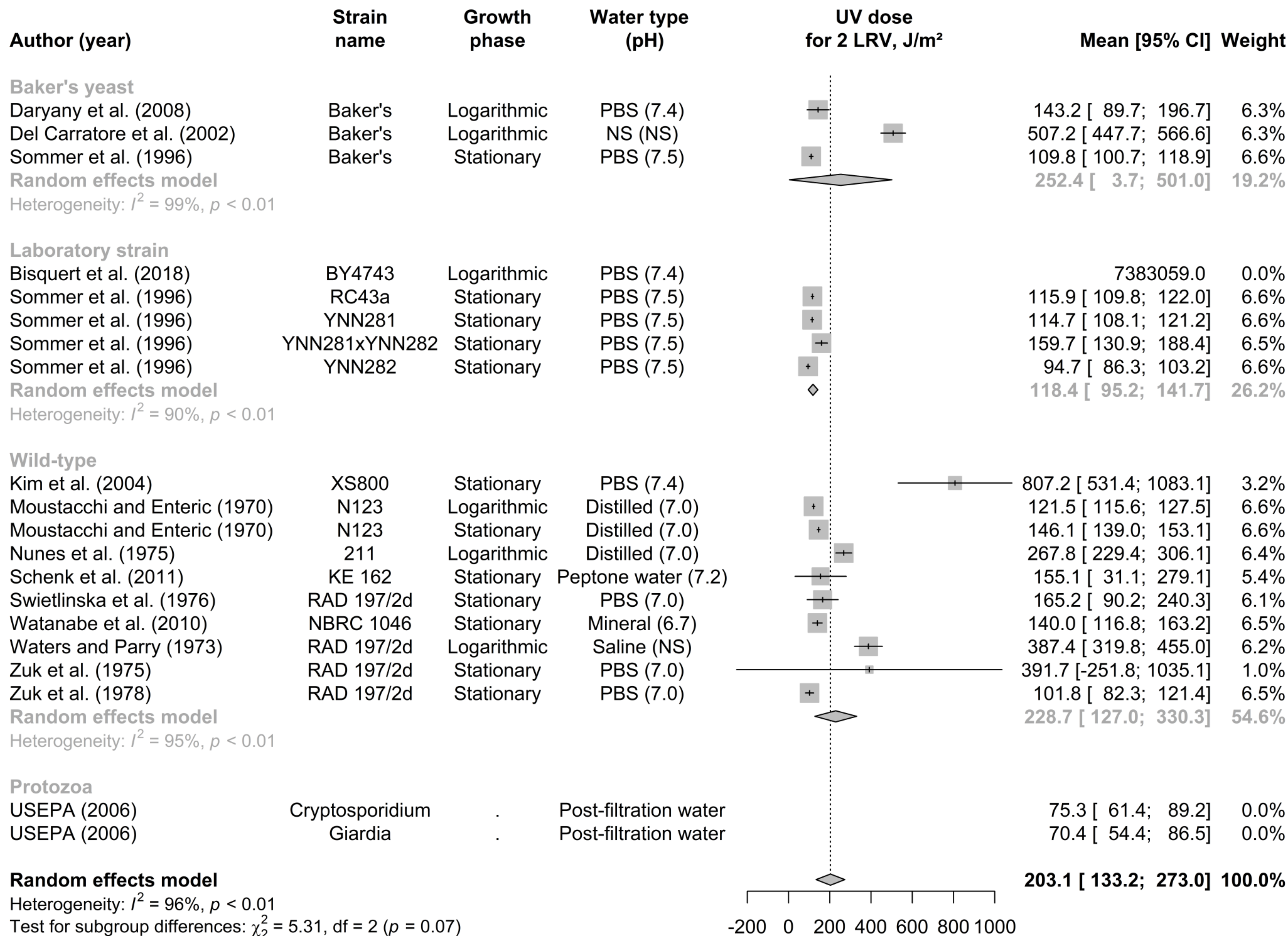


Figure 5.2: Estimates of UV dose required to achieve a $2log_{10}$ microbiological reduction NS = not specified; PBS = phosphate buffered saline). Black vertical lines depict effect estimates; black horizontal lines display 95% CIs for effect estimates. Shaded square areas represent the study weight towards the summary effect estimate using random effects modelling. Diamond shapes depict subgroup and overall summary effect sizes; diamond width represents the 95% CI on the summary effect.

A funnel plot was generated to investigate potential bias in UV dose-response estimates of *S. cerevisiae* (Figure 5.3). Nearly all estimates (16 of 17, or 94.1%) give statistically significant results, with $p < 0.05$ (i.e., a non-zero value for UV dose for 2 LRV was reported). One study[336] had a high standard error on the estimate, which resulted in a CI that included zero and therefore a non-significant result. Under the random effects model[328], the high standard error resulted in a low study weight (1.0%) when the overall effect size was calculated. The majority of estimates (12 of 17, or 70.6%) of UV dose for 2 LRV fell below the overall effect size (i.e., 203.1 J/m^2), with a small number of estimates (5 of 17, or 29.4%) with higher values[280,312,334,336,338], in some cases by more than four times[280]. Therefore, the small number of studies with high effect estimates may bias the overall effect estimate to be higher. Very few estimates (3 out of 17, or 17.6 %) lie within the dashed triangular region, corresponding to the 95% confidence interval of our summary effect estimate. This indicates a wide range of effect estimates yielding highly heterogenous results, which is consistent with our overall forest plot analysis (Figure 5.2).

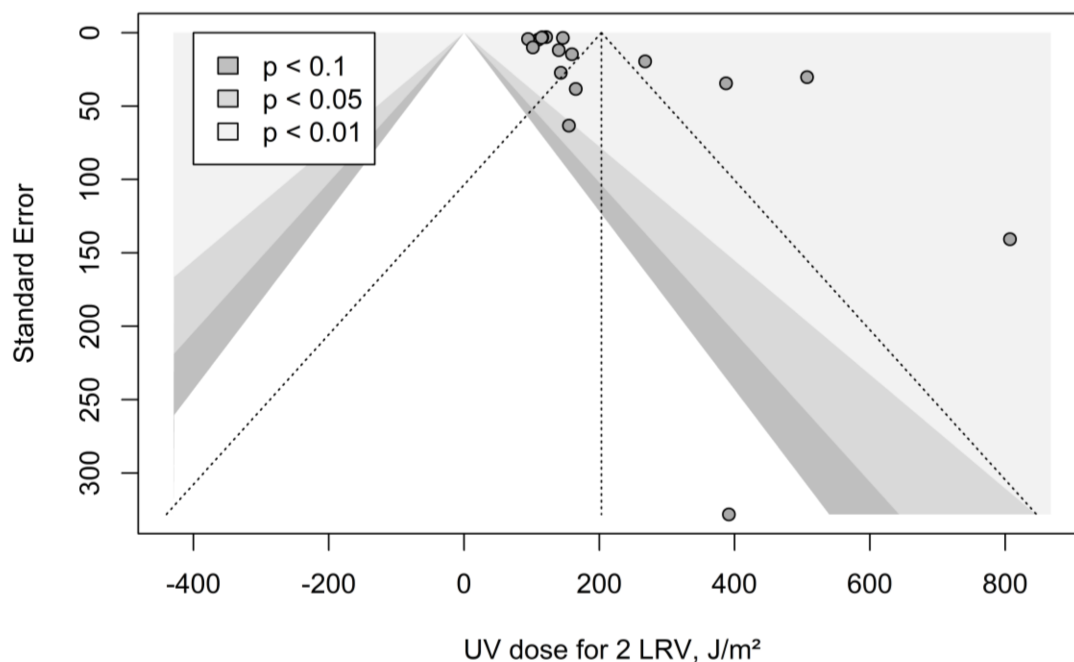


Figure 5.3: Funnel plot to assess bias in UV dose-response estimates of S. cerevisiae. *The vertical dashed line represents the overall summary effect estimate for 2 LRV (i.e., 203.1 J/m^2). The diagonal dashed lines indicate the triangular region within which 95% of study effect estimates are expected to lie in the absence of bias and heterogeneity (i.e., overall summary effect ±1.96×standard error of summary effect). The white triangle represents the expected region for statistically negligible summary effects (i.e., a triangular region centred around zero, signifying no effect), with shaded regions corresponding to statistical significance level of reported summary effects.*

Free chlorine

No summary effect estimate (i.e., chlorine dose for 2 LRV, mg · min/L) was computed for free chlorine because there were only two studies[311,314] with highly disparate effect estimates ($I^2 = 99.9\%$, $p < 0.01$, Figure 5.4). Hays et al.[314] and Buschini et al.[311] both differed in their CT estimates from that of *G. lamblia* ($p < 0.01$ for both), by several orders of magnitude.

Effect size estimates from Hays et al.[314] were somewhat low and had a confidence interval that included zero (Figure 5.4), indicating that *S. cerevisiae* was easily inactivated with free chlorine. Test conditions in Hays et al.[314] were done under either relatively high (8.6) or low (5.3) pH values; the effect size estimates did not significantly differ between the two conditions ($p = 0.62$). There is not a USEPA comparison[344] for *G. lamblia* for pH values below 6.5, but the effect estimate by Hays et al.[314] at pH 8.6 (0.7 mg · min/L; 95% CI -3.0; 4.3 mg · min/L) differs by two orders of magnitude from the corresponding USEPA value for *G. lamblia* at pH 8.5 (49.0 mg · min/L; 95% CI 46.8; 51.2 mg · min/L; Figure 5.4).

The effect estimates from Buschini et al.[311] were several orders of magnitude higher than those reported for *S. cerevisiae* by Hays et al.[314] and by the USEPA for *G. lamblia*[344] under similar pH conditions, indicating that *S. cerevisiae* was more difficult to inactivate with free chlorine. Buschini et al.[311] examined the same *S. cerevisiae* strain under both logarithmic and stationary phase conditions; the effect sizes under the two conditions were statistically different ($I^2 = 98.6\%$, $p < 0.01$).

Chlorine dioxide

Similarly to free chlorine, no summary effect estimate (i.e., dose for 2 LRV, mg · min/L) was computed for chlorine dioxide because there was only one study that examined this disinfectant (Buschini et al.[311], Figure 5.5); in fact the same study that examined dose-response for free chlorine. The chlorine dioxide dose required for $2\log_{10}$ reduction in *S. cerevisiae*[311] was several orders of magnitude larger than that required for *G. lamblia*[344] ($p < 0.01$, Figure 5.5). Buschini et al.[311] examined the same *S. cerevisiae* strain under both logarithmic and stationary phase conditions; the effect sizes for chlorine dioxide under the two conditions were statistically different ($I^2 = 89.8\%$, $p < 0.01$).

Ozone

Two papers were identified[313,318] that examined the dose-response of *S. cerevisiae* to ozone. The effect size (i.e., ozone dose required for $2\log_{10}$ reduction, mg · min/L) by Watanabe et al.[318], 0.1 mg · min/L (95% CI 0.0; 0.1 mg · min/L, Figure 5.6) is on the same order of magnitude as the USEPA estimates for *G. lamblia* at the same temperature (20°C, 0.3 mg · min/L), but the estimates are statistically different ($p < 0.01$).

The other paper examining ozone disinfection[313] gave an effect size (i.e., ozone dose required for $2\log_{10}$ reduction, mg · min/L) that was three to four orders of magnitude larger than that reported by Watanabe et al.[318] (Figure 5.6). Dubeau and Chung[313] examined the dose-response of *S. cerevisiae* at early and late stationary phases of growth – which were not examined by any other study – as well as logarithmic phase. The reported effect size was statistically different for all three growth phases examined (distilled water only, $p < 0.01$). The subgroup estimate for distilled water is also statistically different from that of YEPD nutrient broth ($p < 0.01$). The overall size estimates of Dubeau and Chung[313] were several orders of magnitude larger and statistically different from that for *G. lamblia* (excluding YEPD broth; 20 to 25°C only; $p < 0.01$).

Author (year)	Strain name	Growth phase	Water type (pH)	Temperature, Celsius	Free chlorine dose for 2 LRV, mg•min/L	Mean [95% CI]
Yeast						
Hays et al. (1967)	NS	Stationary	Acetate buffer (5.3)	25		-0.2 [-0.5; -0.0]
Hays et al. (1967)	NS	Stationary	USDA buffer (8.6)	25		0.7 [-3.0; 4.3]
Buschini et al. (2004)	D7 diploid	Stationary	PBS (7.4)	37		7254.3 [6015.5; 8493.1]
Buschini et al. (2004)	D7 diploid	Logarithmic	PBS (7.4)	37		18272.0 [16087.1; 20456.9]
Heterogeneity: I^2 = 99%, p < 0.01						
Protozoa						
USEPA (1991)	Giardia	.	(6.5)	25		23.2 [22.2; 24.1]
USEPA (1991)	Giardia	.	(7.0)	25		27.5 [26.3; 28.6]
USEPA (1991)	Giardia	.	(7.5)	25		33.5 [32.1; 34.8]
USEPA (1991)	Giardia	.	(8.0)	25		40.5 [38.7; 42.3]
USEPA (1991)	Giardia	.	(8.5)	25		49.0 [46.8; 51.2]
Heterogeneity: I^2 = 99%, p < 0.01					0 5000 10000 15000 20000	

Figure 5.4: Estimates of free chlorine dose required to achieve a $2log_{10}$ microbiological reduction. Black vertical lines depict effect estimates; black horizontal lines display 95% CIs for effect estimates.

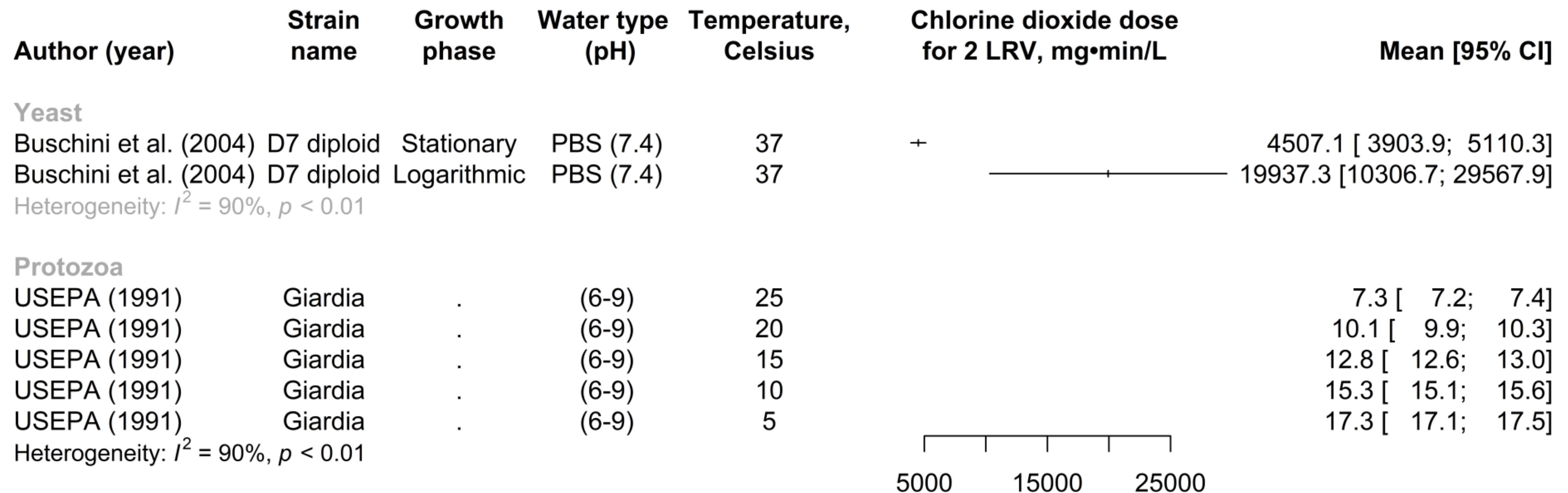
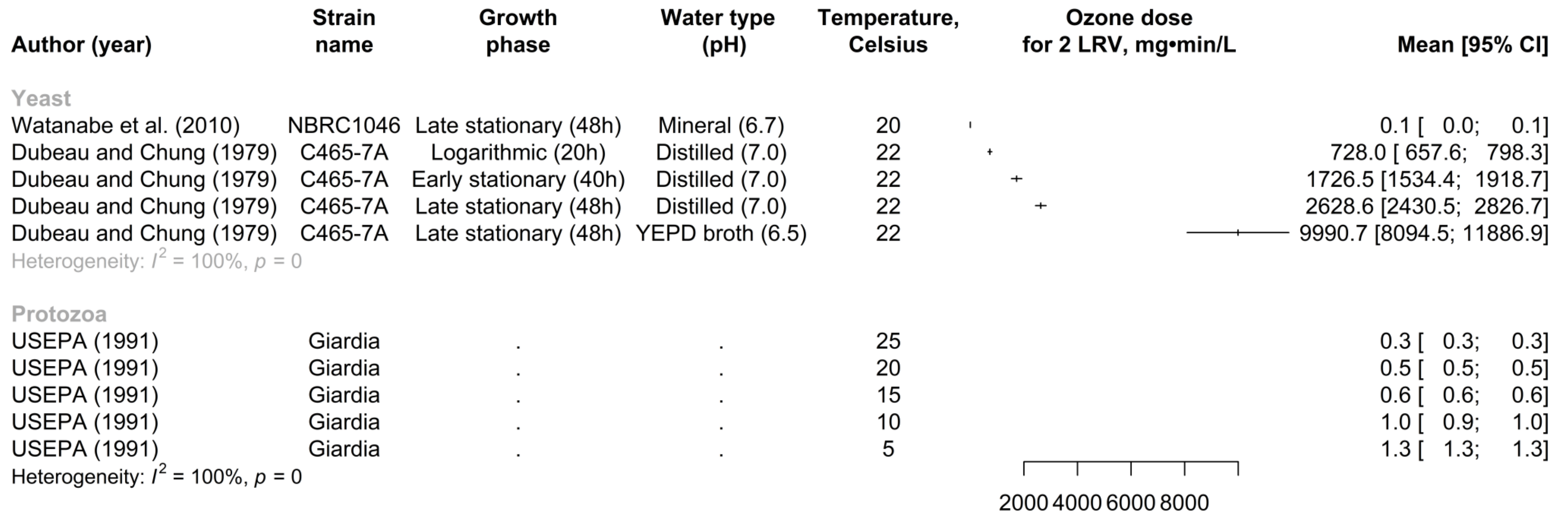


Figure 5.5: Estimates of chlorine dioxide dose required to achieve a $2log_{10}$ microbiological reduction. Black vertical lines depict effect estimates; black horizontal lines display 95% CIs for effect estimates.



Author (year)	Strain name	Growth phase	Water type (pH)	Temperature, Celsius	Ozone dose for 2 LRV, mg•min/L	Mean [95% CI]
Yeast						
Watanabe et al. (2010)	NBRC1046	Late stationary (48h)	Mineral (6.7)	20		0.1 [0.0; 0.1]
Dubeau and Chung (1979)	C465-7A	Logarithmic (20h)	Distilled (7.0)	22		728.0 [657.6; 798.3]
Dubeau and Chung (1979)	C465-7A	Early stationary (40h)	Distilled (7.0)	22		1726.5 [1534.4; 1918.7]
Dubeau and Chung (1979)	C465-7A	Late stationary (48h)	Distilled (7.0)	22		2628.6 [2430.5; 2826.7]
Dubeau and Chung (1979)	C465-7A	Late stationary (48h)	YEPD broth (6.5)	22		9990.7 [8094.5; 11886.9]
Heterogeneity: I^2 = 100%, p = 0						
Protozoa						
USEPA (1991)	Giardia	.	.	25		0.3 [0.3; 0.3]
USEPA (1991)	Giardia	.	.	20		0.5 [0.5; 0.5]
USEPA (1991)	Giardia	.	.	15		0.6 [0.6; 0.6]
USEPA (1991)	Giardia	.	.	10		1.0 [0.9; 1.0]
USEPA (1991)	Giardia	.	.	5		1.3 [1.3; 1.3]
Heterogeneity: I^2 = 100%, p = 0						

Figure 5.6: Estimates of ozone dose required to achieve a $2log_{10}$ microbiological reduction. Black vertical lines depict effect estimates; black horizontal lines display 95% CIs for effect estimates.

5.7 Discussion

5.7.1 Systematic review

Most studies examining the disinfection properties of *S. cerevisiae* are in the topic of food science with *S. cerevisiae* as the target organism of interest because it is a spoilage organism[119,267], as opposed to a surrogate for another organism. Chemical disinfection methods (i.e., chlorine dioxide, free chlorine, or ozone) are not commonly used to disinfect food-related items, perhaps leading to a paucity of work in this area (5 studies total for chemical disinfection methods compared to 13 studies for UV; see Table 5.4).

5.7.2 Meta-analysis

UV

The results are unclear regarding the impact of strain choice or growth phase on effect estimates (i.e., UV dose required for $2\log_{10}$ reduction, in J/m^2). In general, effect sizes are highly heterogenous, even within subgroups according to strain type and growth phase (Figure 5.2; Table 5.5). Finer analyses indicate that some strains respond similarly to UV disinfection (i.e., the RC43a, YNN281 and baker's yeast strains studied by Sommer et al.[316]), while other comparisons between different strains indicate a high degree of heterogeneity and statistical differences (i.e., wild-type *S. cerevisiae* under both logarithmic or stationary phase conditions). In some cases, different studies investigating the same strain under the same growth conditions produced statistically similar effect sizes (i.e., the RAD 197/2d strain studied in stationary phase[336,337,339]), indicating reproducibility; in others, the effect sizes were statistically different (i.e., baker's yeast studied in logarithmic phase[310,312]).

The results are also unclear regarding phase of culture growth. An overall comparison between logarithmic phase and stationary phase subgroups (Table 5.5) showed no statistical difference in effect size, as did subgroup comparisons within wild-type and baker's yeast strains, although finer comparisons did reveal some differences. The only study to examine the same strain under both stationary and logarithmic phases showed statistically differing effect sizes[333]; different studies studying the same strain (RAD

197/2d) also showed statistically different effect sizes between stationary and logarithmic phases[336–339].

Masjoudi et al.[160] have published a compilation of dose-response data for various pathogens and indicators. They include one study examining *S. cerevisiae*, which we have also included in our literature review and meta-analysis[280]. Citing Kim et al.[280] singly, Masjoudi et al.[160] report a UV fluence (dose) of 700 J/m^2 required for 2 LRV, although they do not report the methods used to arrive at this estimate. Based on the same study[280], we calculated the required UV dose for 2 LRV to be 807.2 J/m^2 (95% CI 531.4; 1083.1 J/m^2; Figure 5.2). Although our estimate differs from that of Masjoudi et al.[160], it is within our calculated 95% CI and on the same order of magnitude.

It should be noted that the data published by Kim et al.[280] yields the highest dose estimate for 2LRV of all studies included in our meta-analysis. The 2 LRV dose estimate from Kim et al. (807.2 J/m^2, 95% CI 531.4; 1083.1 J/m^2; Figure 5.2) is nearly four times that of our overall dose estimate for *S. cerevisiae* (203.1 J/m^2, 95% CI 133.2; 273.0 J/m^2; Figure 5.2). Those employing the singular 2 LRV dose estimate by Masjoudi et al.[160] would be led to conclude that the resistance of *S. cerevisiae* to UV disinfection is substantially higher than indicated by the full body of literature as described in the present work.

Masjoudi et al.[160] estimate the UV dose required for $2\log_{10}$ inactivation of *G. lamblia* and *C. parvum* to be 10 J/m^2 (no CI reported) and 24 J/m^2 (95% CI 10; 38 J/m^2), respectively. Other reviews have been published compiling dose-response data for various microorganisms including *G. lamblia* and *C. parvum*[297,345], however these reviews did not include *S. cerevisiae.* Hijnen et al.[297] do not report an estimate for *G. lamblia*, but do report an estimate for 2 LRV of *C. parvum* with polychromatic UV radiation (i.e., a wider wavelength range than 254 nm as in our meta-analysis) to be 20.5 J/m^2.

Qian et al.[345] report modelled dose-response estimates for *G. lamblia* and *C. parvum:* 4.7 J/m^2 and 6.3 J/m^2, respectively for 2 $\log_{10}$ inactivation. This is lower than other estimates noted here[160,297,329]; although this may be because Qian et al.[345] modelled dose-response estimates to 20 LRV with higher doses of up to 1350 J/m^2; our analysis space of 2 LRV is on the low end of their estimates, making comparisons difficult.

Our meta-analysis for UV indicates us that the dose-response of *S. cerevisiae* is statistically comparable to *G. lamblia* (p = 0.36 and 0.47 for comparisons with overall *S. cerevisiae* and Baker's yeast subgroup, respectively; Table 5.6), and *C. parvum* (p = 0.37 and 0.48

for comparisons with overall *S. cerevisiae* and Baker's yeast subgroup, respectively; Table 5.6). However, our *S. cerevisiae* meta-analysis results are heterogenous, and the CI is wide (Figure 5.2), especially regarding the subgroup of baker's yeast, which was only comprised of three studies[310,312,316]. The mean estimate for a 2 LRV dose for *S. cerevisiae* (overall estimate 203.1 J/m^2; Baker's yeast subgroup estimate 252.4 J/m^2; Figure 5.2) is more than double those of *G. lamblia* and *C. parvum* (75.3 J/m^2 and 70.4 J/m^2, respectively; Figure 5.2). *S. cerevisiae* requires more than double the IT dose as *G. lamblia* and *C. parvum* to inactivate microbes by 2 orders of magnitude ($2\log_{10}$). Therefore, we can say that *S. cerevisiae* is a conservative surrogate for *G. lamblia* and *C. parvum* at UV doses typically seen in water disinfection.

Free chlorine

Of the two studies that examined the dose-response of *S. cerevisiae* to free chlorine, one[314] yielded effect sizes (i.e., CT required for $2\log_{10}$ reduction) that was two orders of magnitude lower than that of *G. lamblia*[344], while the other[311] yielded effect sizes two to three orders of magnitude higher than *G. lamblia*[344] (Figure 5.4). Comparison between *G. lamblia* and *S. cerevisiae* is further limited because reported *S. cerevisiae* test conditions differed substantially from those of the USEPA[344] for *G. lamblia*.

Two oddities of note emerged from the free chlorine studies. First, the effect sizes reported by Hays et al.[314] for pH 5.3 and 8.6 were not statistically different ($p = 0.62$), as would generally expected at a high pH compared to a low pH. Under high-pH conditions, the hypochlorous acid-hypochlorite ion equilibrium reaction ($HOCl \leftrightarrow OCl^-$) favours the hypochlorite ion (OCl^-), which is a substantially weaker disinfectant[51]. Therefore, disinfection activity would decrease, resulting in a higher effect estimate under high pH conditions. The second oddity that emerged was that the temperatures used by Buschini et al.[311] were higher than those by both Hays et al.[314] and the USEPA[344]. This would generally result in free chlorine reactions proceeding more quickly and therefore a lower effect estimate[51], but in fact the opposite was the case.

Owing to the highly divergent effect sizes and test conditions, no conclusion can be drawn based on the current literature regarding the suitability of *S. cerevisiae* as a potential surrogate for *G. lamblia* regarding free chlorine disinfection. Further experimental work would be necessary to confirm or repudiate the findings of Hays et al.[314] and/or Buschini et al.[311]. Based on Buschini et al.[311], growth phase conditions have a statistically significant

impact on the dose-response behaviour of *S. cerevisiae* to free chlorine (Figure 5.4), although this finding is from a single study only and would also need to be confirmed or repudiated by further work.

Chlorine dioxide

Only one study[311] examined the dose-response of *S. cerevisiae* to chlorine dioxide disinfection in water; the effect size was two to three orders of magnitude higher than USEPA[344] estimates for *G. lamblia*. The temperature conditions used by Buschini et al.[311] (i.e., at 37°C) hampers comparison to USEPA[344] estimates for *G. lamblia* (i.e., at 25°C). Similar to free chlorine disinfection, at lower temperatures chlorine dioxide disinfection proceeds more slowly[51], resulting in a higher CT required for a given LRV; thus, if conducted at 25°C, the CT estimate by Buschini et al.[311] would be higher than reported at 37°C. Therefore, *S. cerevisiae* would be an even more conservative surrogate for *G. lamblia* at 25°C. However, overall conclusions cannot be drawn regarding the suitability of *S. cerevisiae* as a surrogate for *G. lamblia* for chlorine dioxide disinfection because there is only one study upon which to base conclusions. Again similar to free chlorine disinfection, growth phase conditions did impact the dose-response behaviour of *S. cerevisiae* observed by Buschini et al.[311], although conclusions regarding this are limited to observations within a single study.

Ozone

Two studies were found that examined the dose-response behaviour of *S. cerevisiae* to ozone disinfection (Figure 5.6); one of which[318] yielded an effect size estimate on the same order of magnitude but statistically lower than *G. lamblia*[344]. The other study[313] provided effect estimates that were four to five orders of magnitude higher than that of *G. lamblia* at similar temperatures[344]. Based on the two inconsistent studies included in our meta-analysis for ozone disinfection[313,318], we cannot draw conclusions regarding the suitability of *S. cerevisiae* as a surrogate for *G. lamblia* for ozone disinfection; more laboratory investigation would be needed to investigate this.

Dubeau and Chung[313] conducted testing across three growth phases; all three conditions produced statistically different effect sizes. Like our other chemical disinfection findings, this suggests that growth phase conditions do have an impact on the dose-response

behaviour of *S. cerevisiae*, however this is based on a single study and would need to be confirmed by further work.

5.7.3 General discussion

A suite of validated biological and non-biological surrogates for *C. parvum* and *G. lamblia* (outlined in Section 5.4) are available for various disinfection methods; in our imagining, *S. cerevisiae* could expand this suite and be used in conjunction with some existing field-appropriate surrogates (e.g., turbidity reduction). Other literature has suggested yeast as preferred indicators of disinfection efficacy (i.e., by chlorination in traditional water treatment plants) over coliforms[346]. Yeasts have been studied for use as surrogates in wastewater disinfection efficacy[347]; in most cases, the species of yeast were found to be several orders of magnitude more resistant to chlorine and chloramine disinfection as salmonella, *E. coli* or poliovirus[347,348].

Based on the surrogate selection criteria set by Sinclair et al.[111], our investigation into *S. cerevisiae* as a surrogate for protozoan pathogens fell under the category of "Benchmarking II" (i.e., "Through a literature review, demonstrating that the surrogate possesses the same [experimental] attributes as the target pathogen"). There is no such thing as a perfect surrogate[111], but using *S. cerevisiae* for UV disinfection applications (and possibly chemical disinfection if it is validated) would open the opportunity to investigate water treatment methods *in situ*, providing useful information where there is currently a lack of data[20,41].

Further work is needed to validate and correlate *S. cerevisiae* to protozoan pathogens with respect to chemical disinfection methods (free chlorine, chlorine dioxide and ozone disinfection) due to the paucity of studies in this area. However, limited results reported in the present analysis show that yeast could be a conservative surrogate (Figure 5.4 through Figure 5.6). It would be possible to use *S. cerevisiae* right away to conduct testing in field studies, and as further testing is done, the correlation between *S. cerevisiae* and protozoan pathogens can be established, and findings can be adjusted accordingly.

Other studies[37,281] have successfully validated *S. cerevisiae* as a protozoan surrogate for use in filtration applications and it has been used as a challenge organism to evaluate *in situ* performance of non-potable water treatment systems[39,40]. The use of *S. cerevisiae* as a surrogate for protozoan pathogens would decrease the burden to conduct water treatment-related research (i.e., safety protocols, supplies and cost), which are particularly

burdensome for low- and middle-income countries (LMICs) where there is a need for water-related research and solutions to address Sustainable Development Goal 6 (SDG 6: Clean Water and Sanitation; UN, 2018). The ability to transport *S. cerevisiae* without difficulty due to no requirement for refrigeration and ease of importing across customs (work in low-resource contexts or remote-access areas) also lends it to suitability in LMICs. *S. cerevisiae* could be used to gather previously unavailable data, such as the operation of large-scale or *in situ* systems[41], or remote settings where transportation to a full biosafety-certified laboratory is an issue. For example, *S. cerevisiae* could be used in site-specific DOP studies of large-scale systems[19,245], which are onerous to conduct due to shutdown requirements if conventional surrogates are used.

5.7.4 Limitations

Photoreactivation ("dark repair") following UV exposure is present in most cells[166] and was first discovered as a phenomenon in yeast[350,351]. Although many of the papers produced in our literature review examined photoreactivation[280,332–339,341–343], no data was extracted or analyzed regarding this phenomenon. This is consistent with the approach used in the current catalog of microbe dose-response behaviour to UV disinfection[160], which only looks at the inactivating dose, in accordance with the Ultraviolet Disinfection Handbook[352].

As mentioned above, a summary effect estimate was not computed for any chemical disinfection method due to the paucity of studies available. It is not possible to draw substantive conclusions regarding the suitability of *S. cerevisiae* as a protozoan surrogate for chemical disinfection methods based on the available literature.

Few studies achieved high quality (3 out of 16, or 18.8%; Table 5.4), with descriptive statistics (e.g., 95% CI) and lack of a control sample as the most frequently omitted quality factors. The majority of UV disinfection studies did not report any compensation protocols for spatial heterogeneity of the UV lamp output (e.g., petri factor), as is best practice[143,144], and many studies did not report the UV lamp power or specific wavelength. The omission of information required to calculate UV dose resulted in the disqualification of four papers[332,340,341,343] during the data extraction step; it is unknown what the effect of the inclusion of these papers would be on the summary dose-response estimate for *S. cerevisiae*.

5.8 Conclusion

The overall objective of this systematic review was to evaluate *S. cerevisiae* as a protozoan surrogate in application to disinfection-based drinking water treatment methods. Our results suggest that *S. cerevisiae* is a conservative surrogate for UV disinfection of *G. lamblia* and *C. parvum* at doses typically seen in water disinfection. However, we were not able to draw any substantive conclusions regarding the suitability of *S. cerevisiae* as a surrogate for *G. lamblia* with respect to free chlorine, chlorine dioxide or ozone disinfection methods due to the paucity in available studies. To this end, the study objective was partially addressed as a comparison for chemical disinfection methods was not possible. To fully address the study objectives would require further laboratory investigation. *S. cerevisiae* can be considered as a useful surrogate for protozoan pathogens, which opens up the opportunity to evaluate *in situ* performance of water treatment systems and devices.

5.9 Author contributions

Conceptualization: CZ and CCD; preliminary study identification: SL; methodology: CZ and CCD; literature review, study screening and article selection: CZ, JW and CCD; data extraction: CZ and LPN; numerical modelling: CZ and LPN; meta-analysis: CZ; figure preparation: CZ; writing – original draft: CZ; writing – review and editing: CZ, LPN and CCD; supervision: CCD.

5.10 Funding and disclosure statement

This work was funded by the Natural Sciences and Engineering Research Council of Canada (NSERC). The authors report there are no competing interests to declare.

5.11 Data availability statement

The extracted data and R code used for analysis are available online: https://zenodo.org/badge/latestdoi/671618906

5.12 Supplementary information

Table S5.7: Overall study characteristics - all papers included in literature review.

Paper characteristics											Yeast characteristics						Water characteristics					Data info		
Index	**Reference**	**Disinfection Method**	**Examined in lm analysis**	**Number of points extracted**	**Number of points used in meta analysis**	**number of points used in meta analysis for pivot**	**Study quality (#)**	**Max study quality**	**Study quality, % of max**	**Study quality, bin low: < 70% medium: 71 to 90% high: > 90%**	**Strain name**	**Strain type**	**Growth phase**	**Culture method**	**N_0 [cells/mL]**	**N0 [cells/mL] category**	**pH**	**pH categories**	**water type**	**temp ©**	**UV wavelength [nm]**	**Data type**	**Reps**	**Stat.**
1	Del Carratore et al. (2002)	UV	Y	4	4	4	13	17	76	Medium	bakers	Wild-type	log	Pour plate	10^5	10^5	NS	NS	NS		254	Graph	triplicate	NS
2	Daryany et al. (2008)	UV	Y	10	6	6	15	17	88	Medium	bakers	Wild-type	stationary [1]	Pour plate	10^3.8 - 10^6.6	10^4 10^5 10^6	7.4 [2]	7.1-7.5	PBS		355 266	Graph	triplicate	stddev
3	Bisquert et al. (2018)	UV	Y	2	2	2	12	17	71	Medium	BY4743	Laboratory	log	Pour plate	NS [3]	NS	7.4 [2]	7.1-7.5	PBS		254	Graph	triplicate	stddev
4	Kim et al. (2004)	UV	Y	5	3	3	14	17	82	Medium	XS800	Wild-type	stationary	Pour plate	10^6	10^6	7.4	7.1-7.5	PBS		254	Graph	triplicate	stderr
5	Mijowska et al. (2017)	UV	N	0	0	0	11	17	65	Low	NS	NS	stationary	Pour plate	3x10^6	10^6	NS	NS	saline		254	Graph	duplicate	NS
6	Moustacchi and Enteric (1970)	UV	Y	17	8 9	17	12	17	71	Medium	N123 N123uvs$_2$	Wild-type	stationary log	Pour plate	2-3x10^6	10^6	7	7.0	distilled		253.7	Graph	triplicate	95% CI
7	Nunes et al. (1975)	UV	Y	7	7	7	15	17	88	Medium	211	Wild-type	log	Pour plate	10^5	10^5	7	7.0	distilled		254	Graph	triplicate	95% CI
8	Parry and Parry (1976)	UV	N	0	0	0	9	17	53	Low	rad10 rad2 rad3 rad15 rad50	Laboratory Wild-type	log	Pour plate	2x10^7	10^7	NS	NS	saline		254	Graph	duplicate	NS
9	Zuk et al. (1975)	UV	Y	5	5	5	11	17	65	Low	RAD 197/2d rad3 rad6	Wild-type	stationary	Pour plate	2x10^7	10^7	7.0	7.0	PBS		NS	Graph	duplicate	NS
10	Zuk et al. (1978)	UV	Y	5	5	5	11	17	65	Low	RAD 197/2d rad21 rad22 rad20 rad11 rad19 rad12 rad16 rad14 rad7	Wild-type	stationary	Pour plate	NS	NS	7.0	7.0	PBS		NS	Graph	duplicate	NS
11	Waters and Parry (1973)	UV	Y	8	8	8	13	17	76	Medium	RAD/RAD (197/2d) rad3	Wild-type	log	Pour plate	10^7	10^7	NS	NS	saline		235.7	Graph	duplicate	NS
12	Sommer et al. (1996)	UV	Y	54	9 9 9 9 9 0	45	16	17	94	High	RC43a bakers YNN281 YNN282 YNN281xYNN282 YNN281xYNN282 spores	Laboratory Wild-type	stationary [1]	Pour plate	10^6	10^6	7.5	7.1-7.5	PBS		253.7	Graph	quadruplicate	stderr
13	Swietlinska et al. (1976)	UV	Y	5	5	5	11	17	65	Low	RAD 197/2d rad3 rad6	Wild-type	stationary [1]	Pour plate	2x10^7	10^7	7.0	7.0	PBS		NS	Graph	duplicate	NS
14	Schenk et al. (2011)	UV	Y	5	4	4	15	17	88	Medium	KE162	NS	stationary	Pour plate	5x10^7 - 5x10^8	10^7 10^8	7.2 [2]	7.1-7.5	peptone		253.7	Graph	triplicate	stddev
15	Watanabe et al. (2010)	Ozone UV	Y	19 9	19 9	28	14 15	14 17	100 88	High Medium (Say high for both)	NBRC1046	NS	stationary [1]	Pour plate	10^7 to 10^8 10^5 - 10^6	10^5 10^6 10^7 10^8	6.7	6.5-6.9	mineral		NS	Graph	triplicate	stddev
16	Mucka et al. (2010)	UV	N	0	0	0	15	17	88	Medium	CCM 8191 DBM 272	Wild-type	stationary	Pour plate	10^6 - 10^7	10^6 10^7	NS	NS	saline		254	Graph	triplicate	95 CI
17	Parry (1971)	UV	N	0	0	0	11	17	65	Low	Wild-type U1/U1 U4/U4 E10/E10	Laboratory Wild-type	stationary	Pour plate	10^7	10^7	NS	NS	saline		235.7	Graph	duplicate	NS
18	Parry (1972)	UV	N	0	0	0	10	17	59	Low	E9 E5	Laboratory	log	Pour plate	10^7	10^7	NS	NS	saline		253.7	Graph	duplicate	NS
19	Parry and Parry (1972)	UV	N	0	0	0	11	17	65	Low	Wild-type a/alpha/+ a/alpha/ade20 a/alpha/ade20/+ a/alpha/ade20/ade 21	Laboratory Wild-type	log	Pour plate	10^7	10^7	NS	NS	saline		235.7	Graph	duplicate	NS
20	Buschini et al. (2004)	Free chlorine Chlorine dioxide	Y	14 18	12 16	28	12 12	13 13	92 92	High High	Diploid D7	Laboratory	stationary log	Pour plate	10^8	10^8	7.4	7.1-7.5	PBS	37	NA	Table	triplicate	stddev
21	Hays et al. (1967)	Free chlorine	Y	12	8	8	8	13	62	Low	NS	NS	stationary [1]	Pour plate	4.7*10^7	10^7	8.6 5.3	< 6	USDA buffer Acetate buffer	25	NA	Table	NS	NS
22	Dubeau and Chung (1979)	Ozone [a]	Y	98	92	92	10	14	71	Medium	C465-7A (wild) C430-1D (mutant) G218/7a (mutant) G160/2b (mutant) 197/2d derived (mutant)	Laboratory Wild-type	stationary log	Pour plate	10^7	10^7	7.0 [2]	7.0	distilled	22	NA	Graph	triplicate	NS

[a] *Ozone bubbling rate = 2.1 L/m*

Table S5.8: Quality assessment checklist - all papers included in literature review.

Section number	Section name	Criteria	Description	Bisquert et al. (2018)	Daryany et al. (2008)	Del Carratore et al. (2002)	Kim et al. (2004)	Mijowska et al. (2017)	Moustacchi and Enteric (1970)	Mucka et al. (2010)	Nunes et al. (1975)	Parry (1971)	Parry (1972)	Parry and Parry (1972)	Parry and Parry (1976)	Schenk et al. (2011)	Sommer et al. (1996)	Swietlinska et al. (1976)	Watanabe et al. (2010) - UV	Waters and Parry (1973)	Zuk et al. (1975)	Zuk et al. (1978)	Buschini et al. (2004) - free Cl	Buschini et al. (2004) - ClO2	Hays et al. (1967) - Free Cl	Dubeau and Chung (1979) - O3	Watanabe et al. (2010) - O3
1	General	Peer review	Was the study subject to peer	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1	General	Publication year	Publication newer than 1990	1	1	1	1	1	0	1	0	0	0	0	0	1	1	0	1	0	0	0	1	1	0	0	1
2	Methods - experimental design	Controls	A control sample is used and reported (i.e., dose = 0)	1	1	1	0	1	0	1	1	0	0	0	0	1	0	1	1	0	0	1	1	1	0	0	1
2	Methods - experimental design	Influent concentration	The pre-exposure microbe concentration is reported (approx is OK)	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
2	Methods - experimental design	Water characteristics	Water characteristics are reported (pH, water type (e.g., phosphate-buffered saline), temperature if relevent	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
3	Methods - microbiology	Strain information	Strain name given	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1
3	Methods - microbiology	Preparation methods	Pre-test microbiological prep methods described (e.g., incubation in nutrient broth to	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
3	Methods - microbiology	Enumeration method	Appropriate enumeration methods described?	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1
4	Methods - statistics	Number of data points	0 points if ≤ 2, 1 point if between 3 and 6, 2 points if more than 6 (6 points is the reccomended number by Linden et al (2015 &	0	2	1	1	0	2	2	2	2	1	2	0	1	2	1	2	2	1	1	2	2	2	1	2
4	Methods - statistics	Number of reps	Reps reported (e.g., duplicate or triplicate)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1
4	Methods - statistics	Statistical information	Statistical information reported (e.g., standard deviation or 95% CI on points)	1	1	0	1	0	1	1	1	0	0	0	0	1	1	0	1	0	0	0	1	1	0	0	1
5	Methods - UV only	Yeast strain	At least one non-UV-sensitive strain of yeast is studied [a]	1	1	1	1	1	1	1	1	1	0	1	0	1	1	1	1	1	1	1					1
5	Methods - UV only	UV wavelength	Wavelength of the UV light is reported	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	0	0					
5	Methods - UV only	Collimated beam characteristics	Petri factor, etc are reported or described	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0					
5	Methods - UV only	UV power	The power of the UV light is reported (i.e., W/m2)	0	0	1	1	1	0	1	1	0	0	0	0	1	1	0	1	1	1	0					
5	Methods - UV only	UV dose	The dose of UV light is given for a given LRV, or can be reasonably calculated from the reported information [a]	1	1	1	1	0	1	0	1	0	1	0	1	1	1	1	1	1	1	1					
6	Methods - Ozone only	Ozone bubbline rate	Ozone bubbling rate reported																							1	0
7	Methods - chemical disinfection only	Cl dose	Free chlorine, ozone or ClO_2 concentration reported (i.e., dose can be calculated) [a]																				1	1	1	1	1
			Total	12	15	13	14	11	12	15	15	11	10	11	9	15	16	11	15	13	11	11	12	12	8	10	14

[a] *Mandatory criteria for inclusion in data extraction and meta analysis*

PRISMA 2020 for Abstracts Checklist

Section and Topic	Item #	Checklist item	Reported (Yes/No)
TITLE			
Title	1	Identify the report as a systematic review.	Y
BACKGROUND			
Objectives	2	Provide an explicit statement of the main objective(s) or question(s) the review addresses.	Y
METHODS			
Eligibility criteria	3	Specify the inclusion and exclusion criteria for the review.	Y
Information sources	4	Specify the information sources (e.g. databases, registers) used to identify studies and the date when each was last searched.	Y
Risk of bias	5	Specify the methods used to assess risk of bias in the included studies.	Y
Synthesis of results	6	Specify the methods used to present and synthesise results.	Y
RESULTS			
Included studies	7	Give the total number of included studies and participants and summarise relevant characteristics of studies.	Y
Synthesis of results	8	Present results for main outcomes, preferably indicating the number of included studies and participants for each. If meta-analysis was done, report the summary estimate and confidence/credible interval. If comparing groups, indicate the direction of the effect (i.e. which group is favoured).	Y
DISCUSSION			
Limitations of evidence	9	Provide a brief summary of the limitations of the evidence included in the review (e.g. study risk of bias, inconsistency and imprecision).	Y
Interpretation	10	Provide a general interpretation of the results and important implications.	Y
OTHER			
Funding	11	Specify the primary source of funding for the review.	Y
Registration	12	Provide the register name and registration number.	N/A

Figure S5.7: The PRISMA checklist[307,308] for systematic review abstracts, completed for Section 5.1.

PRISMA 2020 Checklist

Section and Topic	Item #	Checklist item	Location where item is reported
TITLE			
Title	1	Identify the report as a systematic review.	Y - title
ABSTRACT			
Abstract	2	See the PRISMA 2020 for Abstracts checklist.	Y – 5.1
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	Y – 5.3.3
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	Y – 5.3.4
METHODS			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	Y – 5.5.1
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	Y – 5.5.1
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	Y – 5.5.1
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	Y – 5.5.1
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	Y – 5.5.2
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	Y – 5.5.2
	10b	List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	Y – 5.5.2
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	Y – 5.5.2
Effect measures	12	Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results.	Y – 5.5.2
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)).	Y – 5.5.2
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	Y – 5.5.2
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	Y – 5.5.2
	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	Y – 5.5.2
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression).	Y – 5.5.2
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.	Y – 5.5.2
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	Y – 5.5.2
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	Y – 5.5.2

Continued on next page.

PRISMA 2020 Checklist

Section and Topic	Item #	Checklist item	Location where item is reported
RESULTS			
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	Y – 5.6.1
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	Y – 5.6.3
Study characteristics	17	Cite each included study and present its characteristics.	Y – 5.6.2 and SI table S5.7
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	Y – 5.6.2
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots.	Y – 5.6.3
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	Y – 5.6.3
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	Y – 5.6.3
	20c	Present results of all investigations of possible causes of heterogeneity among study results.	Y – 5.6.3
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	Y – 5.6.2
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	Y – 5.6.3
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	Y – 5.6.3
DISCUSSION			
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	Y – 5.7.2
	23b	Discuss any limitations of the evidence included in the review.	Y – 5.7.4
	23c	Discuss any limitations of the review processes used.	Y – 5.7.4
	23d	Discuss implications of the results for practice, policy, and future research.	Y – 5.7.3
OTHER INFORMATION			
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	Y – 5.5.1
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	Y – 5.5.1
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	N/A
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	Y – 5.10
Competing interests	26	Declare any competing interests of review authors.	Y – 5.10
Availability of data, code and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	Y – 5.11

Figure S5.8: The PRISMA checklist[307,308] for systematic reviews, completed for Chapter 5.

Chapter 6: Assessing the real-world performance of point of use water treatment methods: field challenge testing and health risk implications

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6.1 Abstract

Point of use water treatment (POUWT) methods are used in many contexts as the final and sometimes only barrier against waterborne illness. The microbiological performance and therefore potential health protection of POUWT technologies are measured via microbiological challenge testing to obtain a $\log_{10}$ reduction value (LRV). Although laboratory-based challenge testing is a valuable tool to evaluate the performance of POUWT approaches under controlled and replicable conditions, such a controlled environment is not necessarily representative of real-life conditions.

The aim of this work was to provide a framework that could be used to gather and contextualize field-relevant LRVs of POUWT methods, as opposed to idealized laboratory conditions. We sought to develop a novel field challenge test technique to obtain *in situ* LRV data. We conducted a study using field challenge testing in Summer 2021 at the

Sombrio Beach campsite, in the Juan de Fuca Provincial Park in British Columbia (BC), Canada. We recruited 144 participants; the most popular method of water treatment was filtration (79.8%) followed by chemical disinfection (15.4%) and UV disinfection (4.8%). Regarding bacterial reductions, the average LRV in the field was 3.18 (95% CI 2.97; 3.39), lower than the average LRV of 6.11 (95% CI 5.92; 6.31) claimed by respective POUWT device manufacturers. Field-obtained protozoan reductions were more similar to manufacturer claims; 3.84 (95% CI 3.59; 4.08) in the field compared to 3.69 (95% CI 3.43; 3.95) claimed. However, manufacturer-claimed LRVs were supported by third party testing for only 3 out of 22 brands (13.6%) and are therefore potentially speculative.

Using our field LRV data, we sought to compare the health impacts of using laboratory-based vs field-obtained LRV data on health risk assessments via a quantitative microbial risk assessment (QMRA) analysis. For the bacterial pathogen class, calculated health risks based on field-obtained LRVs were substantially higher compared to corresponding manufacturer-claimed LRVs. For the protozoan pathogen class, calculated health risks were similar, due to homogeneity between field-obtained and manufacturer-claimed LRVs. Field challenge testing and subsequent QMRA could aid in validation of specific sanitary inspection items as an evidence-based way to support the inclusion or exclusion of specific criteria into the inspection.

6.2 Keywords

Point of use water treatment (POUWT); microbiological performance, quantitative microbial risk assessment (QMRA).

6.3 Introduction

Clean drinking water is a human right, entitling everyone to water that is of sufficient quality, adequate quantity, accessible, affordable and available when needed[1]. Current estimates find that about 2 billion people lack access to safely managed drinking water services, defined as water coming from an improved source (such as a protected well) that is available on-premises when needed, and free from faecal and priority chemical contamination[13]. The impacts of contaminated water are severe: diarrheal diseases are the third leading cause of child mortality, just behind pneumonia and birth complications[353].

In scenarios where access to clean safe drinking water is lacking, point of use water treatment (POUWT) methods can be used to improve water quality and prevent disease[43]. POUWT methods are one piece of the household water treatment and safe storage (HWTS) approach, which also includes safe water collection, transport and storage, proven to significantly reduce waterborne illness[176,354]. Typical approaches to POUWT include chemical disinfection, most commonly with free chlorine but also to a lesser extent chloramines or chlorine dioxide[19]; ultraviolet (UV) disinfection, including solar disinfection (SODIS)[67] and UV-LED (light emitting diode) technology[69,355]; and filtration, such as by ceramic filters[54] or biosand filters[53,356]. POUWT methods are used in many contexts as the final and sometimes only barrier against waterborne illness, including protection against post-collection contamination[357].

Regardless of the context in which they are intended to be used, the microbiological performance and therefore potential health protection of POUWT technologies are measured via microbiological challenge testing[18,19]. This laboratory-based technique consists of spiking water with virus, bacteria and/or protozoans of interest, or their surrogates, treating that water using the POUWT technology of interest, and enumerating microbes before and after treatment to produce a $\log_{10}$ reduction value (LRV), an order-of-magnitude measure of the ability to remove or inactivate microbes of study[18,19]. LRVs produced via laboratory-based challenge testing are contextualized via the WHO rating scheme by placing the results into the "highly protective", "protective" or "interim" categories[19]. LRVs can also be generally contextualized via health risk assessments using the quantitative microbial risk assessment (QMRA) framework, which has myriad potential applications, for example examination of the trade-off between POUWT efficacy (i.e., LRVs) and adoption during use[31–34,80]. Laboratory-based LRVs have previously been used in application to several QMRA analyses to project health gains expected from using a given POUWT method[30,106,107] or weigh mitigation strategies for climate change-related increases in waterborne illness, including POUWT[105].

Although laboratory-based challenge testing is a valuable tool to evaluate the performance of POUWT approaches under controlled and replicable conditions, such a controlled environment is not representative of the conditions under which POUWT methods are used[20] - even if test conditions are designed to mimic real use conditions, for example using challenge water of poor quality[19]. Posited reasons for the "laboratory versus field" LRV discrepancy include poor or variable source water quality[24,28,81,103]; lack of access to local

supply chains or other difficulties for repair or replacement of technology components[24,83,102]; variable manufacturing processes, especially regarding ceramic water filters[82]; improper maintenance practices[25,82]; unfavourable use conditions[24,81] and/or inappropriate post-treatment water handling[21,83]. Laboratory-based challenge testing can overestimate the LRVs of POUWT methods in comparison to real use conditions[19,20]; consequently, QMRA analyses based on laboratory data could be inaccurate. An approach called "field challenge testing" has been proposed to fill the data gap regarding field performance of POUWT methods[20], thereby reflecting the influence of the contexts in which they are used, as opposed to idealized laboratory conditions. Under the field challenge testing approach, challenge water would be spiked with food-safe surrogate organisms outside a laboratory setting to test POUWT techniques under actual use, to satisfy logistical and safety requirements for use outside a laboratory.

The overall aim of this work was to provide a framework that could be used to gather and contextualize field-relevant LRVs of POUWT methods. This aim was approached via two-fold objectives. First, we sought to develop and implement novel *in situ* challenge test protocols of POUWT methods, to obtain *in situ* LRV data. Second, we sought to compare the health impacts of using laboratory-based vs field-obtained LRV data on health risk assessments via a QMRA analysis.

6.4 Methods

6.4.1 Overview

The field-based LRVs in our study were obtained via a novel study of field challenge testing conducted in Summer 2021 at the Sombrio Beach campsite, part of the traditional territories of the Pacheedaht First Nation, in the Juan de Fuca Provincial Park in British Columbia (BC), Canada. The study context was dictated by COVID-19 travel restrictions; backcountry conditions necessitate the use of POUWT devices to provide clean drinking water. It was neither feasible nor ethical at the time of our study to visit low-resource settings in which POUWT technologies are more widely used[43]; the field challenge test technique can be applied to other contexts where POUWT devices are used longer term. Manufacturer-claimed LRVs were assumed to be laboratory-based, and we compared these to our field-obtained LRVs via our QMRA health risk analysis. Despite that, in many cases, manufacturer claims may or may not be based upon robust laboratory study (see

Section 6.6.3), this comparison is instructive and provides a framework upon which future laboratory and field-based challenge tests can be situated.

6.4.2 Field challenge testing

Field setting

The novel field challenge test technique was implemented in Summer 2021 at the Sombrio Beach campsite, which is accessible via car and is an entry/exit point located approximately halfway through the 47-km Juan de Fuca backcountry hiking trail. Backcountry campers were approached for one-on-one surveys, which consisted of a field challenge test of the participants' own water treatment device using food-safe spike microorganisms; a questionnaire, a 100 mL grab sample of the participants' drinking water, and a hand rinse sample measuring microbiological hand contamination. The workflow of our fieldwork and surveys is depicted in Figure 6.1. The questionnaire, grab sample and hand rinse results will be presented in a future publication and are outside the scope of the current objectives. The field survey received University of Victoria ethical approval (#21-0092) and BC Parks authorization (#98700-20). Sombrio Beach is located on the traditional territories of the Pacheedaht First Nation[49]; we contacted the Nation prior to fieldwork and followed up after fieldwork to share water quality monitoring results. Field trips were conducted from May to August 2021 in 3-night trips, with 3 to 17 participants interviewed per field trip. A total of 144 participants were recruited, with 141 (97.9%) completing the questionnaire and 129 (89.6%) completing the field challenge test of their POUWT device (Figure 6.3).

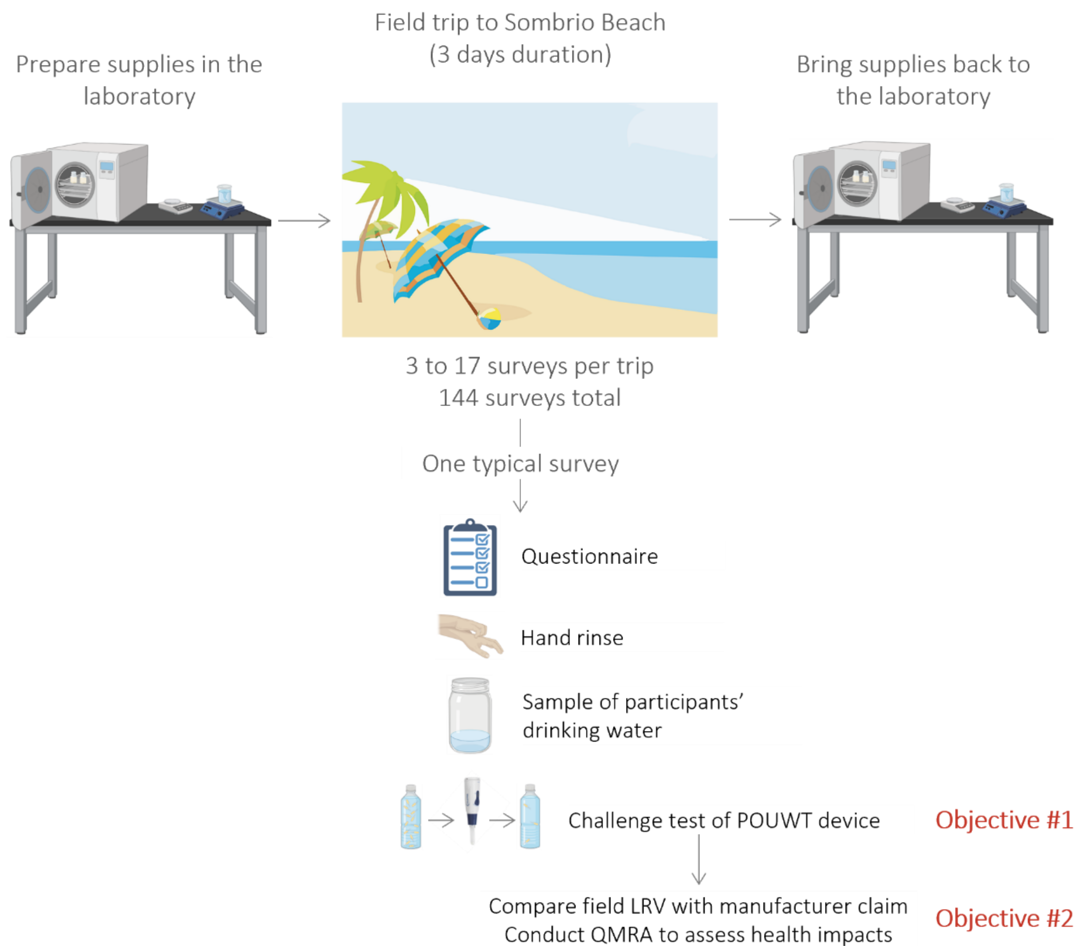

Figure 6.1: Workflow of fieldwork and surveys, with study objectives.

Test water preparation

Probiotic *Escherichia coli* (*E. coli*, strain name Nissle 1917, or EcN, brand name Mutaflor®, Pharma-Zentrale GmbH, Herdecke, Germany) and baker's yeast (*S. cerevisiae*, Traditional Active Dry Yeast, Fleishmann's® Yeast, Fenton, MO, USA) were used for spike microbes as surrogates for bacterial and protozoan pathogens, respectively (see Chapter 3 through Chapter 5). EcN dry powder capsules and jars of *S. cerevisiae* were kept refrigerated once received until field trips, when EcN capsules and aliquots of *S. cerevisiae* were placed in hard-sided coolers (Coleman 8.5 L hard cooler, The Coleman Company, Chicago, IL, USA) containing cubed ice for the duration of each field trip until use. Once taken into the field, EcN capsules and *S. cerevisiae* powder were not used on another trip, and tests were conducted before stated product expiry dates.

The test water used for field challenge testing in this work was taken from Sombrio Creek, at the Sombrio Beach study site, and was prepared daily in 10 L batches. Two capsules of EcN were externally sterilized by wiping the outside with 70% ethanol (Commercial Alcohols, Brampton, ON, Canada) and allowed to dry before aseptically opening and carefully depositing the powder contents into 100 mL of sterile buffered, quarter-strength isotonic Ringers solution (Oxoid Ltd., England, United Kingdom). The mixture was shaken vigorously by hand for a minimum of one minute to dissolve the powder and added to the 10 L test water. Aliquots of 0.25 g of *S. cerevisiae* were prepared in the laboratory before each field trip and stored in an autoclaved 15 mL Falcon tube (Falcon™, Fisher Scientific, Waltham, MS, USA) to take into the field. One 0.25 g aliquot was emptied in to a second 100 mL sterile Ringers solution and shaken vigorously by hand for a minimum of one minute to dissolve the yeast. A 20 mL portion of the yeast-Ringers mixture was aseptically transferred to the 10 L test water. The test water containing EcN and *S. cerevisiae* was vigorously shaken by hand for a minimum of one minute to mix and kept on ice until use, a maximum of 4 hours. Test water was enumerated before challenge tests (described below) in triplicate once per field trip, and singly for all other batches, due to time and supply constraints.

Challenge test with study participants

During one-on-one surveys, participants were asked to treat a 1 L sample of test water as they normally would their own water, using their own PoU water treatment device. The 1 L sample was drawn off from the bulk 10 L challenge test water volume via a spigot into a pre-prepared sterile 1 L polypropylene Nalgene bottle (Economy Wide-Mouth Plastic Bottle, HDPE, Cole-Parmer, Vernon Hills, Illinois, USA). The 1 L test water was given to the participant with a reminder that they will not drink the test water. For treatment methods where water is treated within a water bottle (e.g., chlorine tablets or UV-LED disinfection with SteriPen), the participant was asked to treat the water within the Nalgene as if they were using their own water bottle. All treated challenge test water was enumerated singly (methods described below). For UV-LED disinfection methods, such as SteriPen, participants were instructed to clean their device after the challenge test by first rinsing the outside with treated high-quality water (e.g., tap or bottled water, provided by the researchers), then wiping all outside surfaces with 70% ethyl alcohol (EtOH; Commercial Alcohols, Brampton, ON, Canada).

For chemical disinfection methods, participants prepared and added their disinfecting agent (e.g., chlorine tablets or liquid drops) to the water as they normally would, then were asked how long they typically waited before consuming the water. If the specified contact time was longer than the interview (typically approximately 10 minutes), participants were permitted to collect their participation incentive and depart before the contact time had concluded, while the sample was kept in a covered area at ambient temperature to simulate normal transport within a backpack. At the conclusion of the specified contact time, two 100 mL Whirl-Pak bags were filled with treated test water: one containing chlorine quenching agent and one without (Whirl-Pak 4 oz. Thio Bag B01040 and Whirl-Pak 4 oz. standard sample B00679, respectively, both of Nasco Sampling, Madison, WI, USA). The quenched sample was kept on ice in a cooler until processing for enumeration (maximum 1 hour, see below), and the non-quenched sample was immediately processed for temperature (Bel-Art Enviro-Safe thermometer, ITM Instruments Inc., Vancouver, BC, Canada), pH (test strips 0-14, VWR Chemicals, Radnor, PA, USA), and free and total chlorine concentrations (Pocket Colorimeter II, Hach Company, Colorado, USA).

For those using filtration treatment methods, participants were asked to process 1 L of test water as they normally would. The researchers waited until approximately half of the 1 L test water had been passed through the filter to flush any remaining water from previous use, then filled a non-quenched 100 mL Whirl-Pak bag with effluent mid-stream. The sample was kept on ice in a cooler until processing for enumeration (maximum 1 hour, see below). After the test water was treated, participants were instructed to flush and clean their filters by first processing another 1 L of treated high-quality water (e.g., tap or bottled water, provided by the researchers), rinsing the outside with high-quality water, then wiping all outside surfaces with 70% EtOH. The cleaning approaches aimed to reduce residual EcN and *S. cerevisiae* to below background levels (i.e., less than *S. cerevisiae* numbers typically found on fresh fruits, 10^3-10^5 cells/cm^2 [358]). Participants were also offered to refill their personal water bottles from the researchers' supply of high-quality water, to avoid participants spending extra time treating more water after the interview. If there was any unused spiked challenge test water at the end of the day, it was poured into the ocean where Sombrio Creek terminates.

Enumeration methods

For EcN enumeration, we used field membrane filtration techniques similar to those for the WHO/UNICEF Joint Monitoring Programme[113]. Samples were filtered onto 0.45 µm pore size membranes (Microfil Funnel and Filter, Millipore, Burlington, MA, USA) via vacuum pressure manifold (EZ-Fit Manifold, 1-place, Millipore, Burlington, MA, USA), thus isolating presumptive bacteria on the filter. The filter was removed and placed in a single-use petri dish on top of a selective nutrient media for *E. coli* (m-ColiBlue broth, Hach Company, Colorado, USA Hach). The petri dish was incubated for 24 hours at approximately 35°C[138] using a hot-water bottle incubator[359] because the field site lacked electricity. After incubation, each blue colony, measured as a colony forming unit (CFU), was enumerated by visual inspection and assumed to have originated from a single bacterium[113]. If necessary (i.e., for test water before filtration), serial 10-fold dilutions were carried out prior to plating using buffered, quarter-strength isotonic Ringers solution (pre-prepared in the laboratory). For post-challenge test samples, and blank samples, 100 mL of effluent water was plated. The lower limit of detection for plated samples was 1 CFU per volume of water plated.

Regarding yeast quantification, the SimPlate method for Yeast and Mold Colour Indicator (Y&M-CI) method was used for the detection and quantification of yeast[117]. SimPlate employs Binary Detection Technology, equating a colour change in plated sample "wells" in response to yeast in the medium, analogous to the Most Probable Number (MPN) technique used for detection of *E. coli*[360]. Test water samples were mixed with the pre-sterilized Y&M-CI powder medium, then poured into the SimPlate disc containing 84 wells. The discs were incubated for 63-72 hours at 22-25°C[117] using a separate hot-water bottle incubator[359]. Samples collected on the last day of fieldwork were kept in the hot-water bottle incubator until return to the University of Victoria, when they were transferred to separate laboratory incubators (i.e., one at 25°C and one at 35°C; Forced Air Microbiological Incubator 6.3CF, VWR, Radnor, Pennsylvania, USA) for the remainder of the incubation time. After incubation, wells that had turned colour were enumerated by visual inspection, and this count was converted to Normal Counting Range (NCR, analogous to MPN) via reference tables provided by Millipore[117]. The SimPlate Y&M-CI technique has been validated for use against conventional agar plating methods[118,119] and is more appropriate for low-resource field contexts due to the pre-packaged sterile materials it uses, as well as the lack of requirement for reagent

refrigeration[188]. If necessary (i.e., for test water before filtration), serial 10-fold dilutions were carried out prior to plating using buffered, quarter-strength isotonic Ringers solution (pre-prepared in the laboratory). For post-challenge test samples and blank samples, 10 mL of effluent water was plated. The lower limit of detection for plated samples was 1 NCR per volume of water plated. Resulting arithmetic mean LRVs were tabulated, grouped by POUWT brand, treatment type and spike organism as appropriate.

For quality control, once per trip we processed negative control tests, or "blanks". For membrane filtration (EcN enumeration), 100 mL of pre-sterilized Ringers was processed; for SimPlate (*S. cerevisiae* enumeration), 10 mL of pre-sterilized Ringers was processed (i.e., the required volume of the SimPlate disc).

Manufacturer-claimed LRVs for comparison

For each POUWT device studied in the field, corresponding manufacturer-claimed LRVs were obtained from the manufacturer's website, and/or third-party publications found by searching Google and Google Scholar with the device and manufacturer names. A summary of POUWT brands included in this study and their respective manufacturer claims is presented in Table 6.2. POUWT devices for which field data was collected but manufacturer claims were not available were excluded from the comparitave analysis (Figure 6.3), for a total of n = 104 available comparisons.

6.4.3 QMRA

Overview

We built a QMRA model to simulate pathogen dose and corresponding health risks via drinking water ingestion for a 7-day backcountry hiking trip, using the framework of Haas et al.[361]. Hypothetical hikers were presumed to treat and refill their water three times per day, for a total of 21 water refills per 7-day trip, based on the assumption that hikers would stop to refill their water in the morning, midday and evening. Each given POUWT method was assumed to have the same LRV across all 21 refills in a trip. Point-value LRVs (either field-based gathered via field challenge testing, or corresponding manufacturer claims) were applied across each 21-refill (7-day) backpacking trip to simulate either a field-LRV or manufacturer-LRV scenario. The simulated 7-day trips were described by a combination of raw water concentration, point-value LRV, drinking water consumption,

pathogen dose-response, probability of illness given infection, and estimated DALY weighting per illness (described in detail below).

Conceptually, two hiking trips were simulated for each point-value LRV obtained in the field: one using the field-obtained LRV and one using the corresponding manufacturer-claimed LRV, with all other model inputs held equal (Figure 6.2). This facilitated direct comparison of end points between the two "trips" and eliminated error in attempting to fit a probability density function (PDF) to field-based LRVs, as for some treatment categories there was a paucity of data from which to do so (Table 6.2).

Our QMRA analysis was divided into two steps (Figure 6.2): First, we calculated endpoint values for parallel simulated 7-day backpacking trips (one using a point-value LRV_{field} and one using the manufacturer claimed LRV for the given POUWT device used, $LRV_{manufacturer}$, with all other inputs held equal). Second, we compiled the pairwise endpoint datasets over the range of point-value LRVs (n = 104 comparisons; Figure 6.3). Each parallel 7-day backpacking trip was simulated 10,000 times using a Monte Carlo (MC) sampling method with the seed set to 123. Given 104 manufacturer/field LRV pairs, we produced a total of 1,400,000 MC pairwise comparisons.

Field challenge test and manufacturer-claimed LRV data were managed using Microsoft Excel; QMRA modelling was conducted using RStudio version 2023.03.1; additional open-source libraries were used: Tidyverse (version 2.0.0), Fitdistplus (version 1.1-11), Patchwork (version 1.1.2), gghighlight (version 0.4.0), mc2d (version 0.1-22), Rstatix (version 0.7.2), Readxl (version 1.4.2) and Writexl (version 1.4.2).

Reference pathogens

The reference pathogens we selected were *Campylobacter jejuni* (*C. jejuni*) and *Giardia lamblia* (*G. lamblia*) as representative of the bacterial and protozoan pathogen classes, respectively. A field-appropriate viral surrogate has not yet been identified[20], and therefore we excluded viral surrogates from the scope of our study based on the inability to gather field data regarding viral LRVs (see Section 6.6.3).

C. jejuni was selected as the bacterial reference pathogen on the basis that it is used to establish the performance targets set by the WHO[19] for POUWT devices (i.e., "interim", "protective" and "highly protective" performance categories). It is also one of the pathogens most commonly associated with enteric drinking water outbreaks in Canada[362] and is a

conservative reference pathogen due to its persistence in the environment and high infectivity[134]. *G. lamblia* was selected as the protozoan reference pathogen due to the relatively high incidence of confirmed giardiasis among backcountry recreationists[44] and common association with enteric drinking water outbreaks in Canada[362].

Raw water quality

Pathogen prevalence (PP) and raw (pre-treatment) water concentration (C_{raw}) inputs were stochastic variables based on published data for Canadian surface waters (Table 6.1). Water quality monitoring data from the San Juan River watershed[363] was used for *G. lamblia*, a location which has a similar level of human impact and is near the Sombrio beach field study area on Vancouver Island, Canada. Water quality monitoring data for *C. jejuni* was not available for analogous areas in geographical proximity to Sombrio Beach; we used surface water quality monitoring data gathered from provincial parks in Ontario, Canada[364]. Non-detect pathogen concentrations (C_{ND}) were based on the reported lower detection limit (LDL) using a uniform distribution from zero to the LDL[201] (Table 6.1).

Treatment LRVs

Field-based LRVs were obtained using the field challenge test methods described above; see also above for details on obtaining manufacturer-claimed LRVs. In cases where more than one LRV estimate was published for a given POUWT device, the relevant third-party LRV was used for QMRA if applicable, as opposed to the manufacturer claim. LRVs for bacterial surrogates EcN (i.e., probiotic *E. coli*) and *S. cerevisiae* (i.e., baker's yeast) were used directly as LRVs for *C. jejuni* and *G. lamblia*, respectively.

S. cerevisiae has been validated as a suitable surrogate for *G. lamblia* with respect to filtration processes[37,38] and UV disinfection (Chapter 5). There is a paucity of data regarding chlorine disinfection (Chapter 5) however work is currently underway to address that knowledge gap (data unpublished). EcN has not been validated as a surrogate for *C. jejuni* (see Section 6.6.3); however, *E. coli* spp. are recommended as alternatives by the WHO[19]. Laboratory challenge testing is commonly carried out using *E. coli* and subsequently contextualized via the WHO star ratings, which were designed based on *C. jejuni* as the bacterial reference pathogen[19].

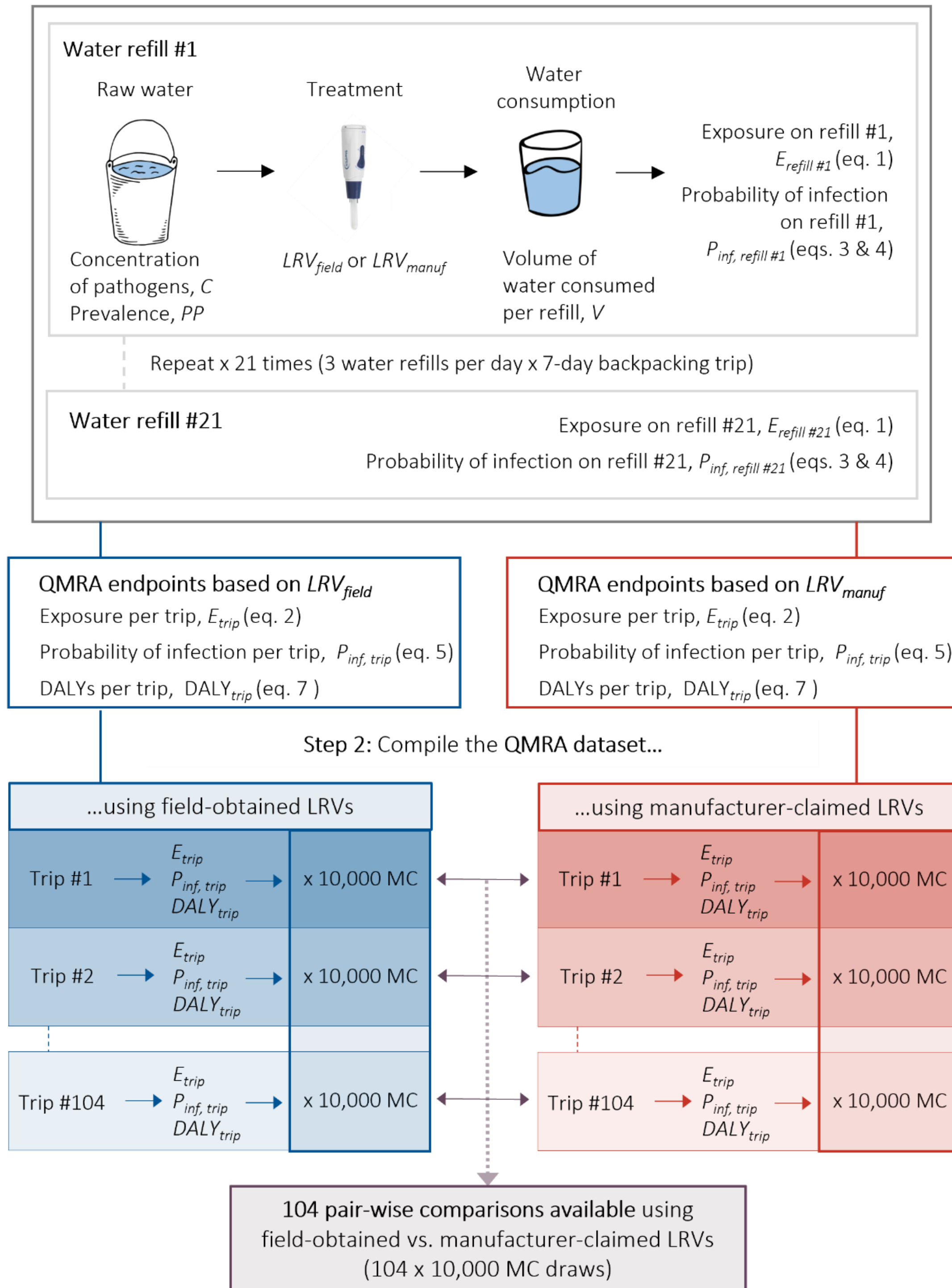

Figure 6.2: Outline of the QMRA model used to simulate pathogen dose via water ingestion for two parallel 7-day backcountry hiking trips, using field-obtained and manufacturer-claimed LRVs, with all other inputs held equal.

Although it is well established that adoption (also referred to as "adherence" or "compliance", with "adoption" as the preferred language per Levy[186]) is a key aspect of the heath protection conferred to the user by a given POUWT device[31,32,34,107], we opted to exclude this aspect from our model. This was for threefold reasons: first, we wanted to use the simplest possible QMRA model to fit our objectives to avoid unnecessary assumptions and associated error. Second, the objective of our work was to compare the calculated health risks using field-generated LRVs versus laboratory-generated LRVs, and any adoption parameter would be equal in both parallel scenarios, resulting in a constant baseline fraction of hikers becoming ill due to non-treatment. This would skew risk estimates upwards for both scenarios equally, but the skew would be arbitrary due to the comparative nature of our objectives. Third, although we did ask study participants about their adoption of POUWT (i.e., by asking "*On this trip, have you drunk any untreated water?*"), it seems likely that they under-reported incidences of drinking non-treated water[365–367] so we do not have a relevant estimate of adoption to apply to our model. Study recruitment was limited to those who were using a POUWT method to treat their water for drinking, so individuals who did not treat their water for drinking at all during their hiking trip were excluded – of whom there were handful that we interacted with over the fieldwork period, albeit anecdotally.

Drinking water consumption

As part of the questionnaire, participants were asked "*On this trip, approximately how much water do you drink per day? (liters)".* Although the results and analysis for the questionnaire as a whole are excluded from the present scope of work, we used participants' responses to this specific question as the basis for modelled drinking water consumption. Participant responses were fitted to a normal PDF having $\mu = 2.45$ L/day, $\sigma = 1.049$ L/day ($n = 141$ responses; Figure 6.3). Water refill volumes (3 refills per day; Figure 6.2) were drawn from a normal distribution having $\mu = 0.82$ L/refill, $\sigma = 0.53$ L/refill (i.e., one-third of the questionnaire-based normal distribution values), with a lower limit of 0.25 L/refill to represent the assumption that hikers are unlikely to refill and drink less than 0.25 L of water at a time (Table 6.1).

End points

The first selected endpoint was the number of pathogens ingested via drinking water over the simulated 7-day backpacking trip (pathogen dose per trip, D_{trip}). Pathogen dose was

calculated based on raw water concentration, treatment LRV and drinking water consumption, using Equations 6.1 and 6.2, detailed in Table 6.1.

The second selected endpoint was the probability of infection over the simulated 7-day backpacking trip ($P_{inf,\ trip}$). We characterized the probability of infection following ingestion of a dose of *C. jejuni* (i.e., following ingestion of water in one refill) using a Beta-Poisson approximation (Equation (6.3)[368], and of *G. lamblia* using the exponential model (Equation 6.4) by Rose et al.[269] as recommended by the WHO[134]. The probability of infection resulting from the 7-day hiking trip was found based on the calculated probability of infection over the 21 simulated refills within the 7-day trip, per Equation 6.5 (Table 6.1).

The third and final endpoint we selected was the expected DALY resulting from the 7-day hiking trip ($DALY_{trip}$). Expected DALYs were calculated based on the stochastic probability of illness given infection with each respective reference pathogen[362,369] (Equation 6.6) and point-estimate DALY weightings of the expected DALYs per illness[134,370] (Equation 6.7). The endpoint in this case was acute gastrointestinal illness (AGI), the most common outcome of illness with *C. jejuni* and *G. lamblia*[134]. A sensitivity analysis of all three endpoints was carried out by calculating pairwise Spearman's rank correlations between stochastic inputs and health risk endpoints.

End point comparisons

As stated above, in each MC simulation, endpoints were calculated for two conceptually parallel hiking trips: one trip using the field-obtained device LRV, and the other trip using the corresponding manufacturer-claimed LRV, with all other inputs held equal (Figure 6.2). Endpoints were compared for each set of paired trips using field-obtained versus manufacturer-claimed LRVs (n = 104 pairs, Figure 6.3). Dose (D_{trip}), probability of infection (P_{inf}) and DALY ($DALY_{trip}$) endpoints were compared by subtracting pairwise endpoints (Table 6.1), to calculate differences in dose (ΔD_{trip}, Equation (6.8), probability of infection (ΔP_{inf}, Equation (6.9) and DALYs ($\Delta DALY_{trip}$, Equation 6.10).

Table 6.1 Detailed description of QMRA inputs used in this work.

Parameter	Symbol (units)	Distribution (input variables) or formula (calculated variables)	Explanation
Raw water pathogen concentration	C_{raw} (CFU or oocysts per L)	*Campylobacter*: lognormal PDF with μ = 19.4, σ = 22.2 *Giardia*: lognormal PDF with μ = 6.40 · 10^{-3}, σ = 7.58 · 10^{-3} Each water refill had a probability (1-*PP*) of a non-detect concentration C_{ND}	Water quality of provincial parks surface water in Ontario, Canada; modelled by Pintar et al.[364]. Published surface water quality monitoring data of the San Juan River watershed (Vancouver Island, British Columbia)[363]; modelled by the authors (see above).
Pathogen prevalence – the probability that any given water refill is contaminated with the organism of interest	*PP* (%)	*Campylobacter:* PERT PDF with min = 0%, mode = 25.0%, max = 57.8% *Giardia*: point estimate = 14.3%	Surface water prevalence for small water systems in Canada[362]. Published surface water quality monitoring data of the San Juan River watershed (Vancouver Island, British Columbia)[363].
Non-detect pathogen concentration in raw water	C_{ND} (CFU or oocysts per L)	*Campylobacter:* Uniform PDF with min = 0, max = 10 *Giardia*: Uniform PDF with min = 0, max = 0.01	Based on the LDL[201] of 1 CFU/100 mL and 1 oocyst/100 L for *Campylobacter*[364] and *Giardia*[363], respectively.
Log reduction value	LRV (unitless)	*Campylobacter & Giardia*: vector of point values	Field-gathered challenge test data or published manufacturer claims (see Figure 6.2).
Drinking water consumption per refill	*V* (L)	Truncated normal PDF with μ = 0.816 L, σ = 0.350 L, lower limit = 0.25 L	Field-gathered questionnaire data modelled by the authors (see above).
Dose per n^{th} refill	$D_{refill\ n}$ (CFU or oocysts per refill)	$D_{refill\ n} = C_{raw} \cdot 10^{-LRV} \cdot V$ (6.1)	Assume 3 water refills per day on a 7-day backpacking trip, *n* = 21.
Dose per 7-day backpacking trip	D_{trip} (CFU or oocysts per trip)	$D_{trip} = \sum_{1}^{n=21} D_{refill\ n}$ (6.2)	Haas et al.[361]

Probability of infection per n^{th} refill	$P_{inf,refill\ n}$ (unitless)	*Campylobacter*: $P_{inf,refill\ n} = 1 - \left(1 + D_{refill\ n} \cdot \frac{2^{\frac{1}{\alpha}} - 1}{N_{50}}\right)^{-\alpha}$ (6.3) where $\alpha = 0.144$ and $N_{50} = 890$ *Giardia*: $P_{inf,refill\ n} = 1 - e^{-r \cdot D_{refill\ n}}$ (6.4) Where $r = 0.0199$	Approximation of the Beta-Poisson model[368]. Exponential model[269], recommended by the WHO[134].
Probability of infection per 7-day backpacking trip	$P_{inf,trip}$ (unitless)	$P_{inf,trip} = 1 - \prod_{1}^{n=21}(1 - P_{inf,refill\ n})$ (6.5)	Haas et al.[361]
Probability of illness given infection	$P_{ill\ \mid\ inf}$ (unitless)	*Campylobacter*: uniform PDF with min = 0.1, max = 0.6 *Giardia*: uniform PDF with min = 0.2, max = 0.7	USEPA[369]; used by Murphy et al.[362]. Endpoint is acute gastrointestinal illness (AGI).
Probability of illness per 7-day backpacking trip	$P_{ill,trip}$ (unitless)	$P_{ill,trip} = P_{inf,trip} \cdot P_{ill\ \mid\ inf}$ (6.6)	Haas et al.[361]
DALY weighting	DW (DALYs per illness)	*Campylobacter:* point estimate = $4.6 \cdot 10^{-3}$ *Giardia*: point estimate = $1.7 \cdot 10^{-3}$	WHO[134] Health Canada[370]. Endpoint is acute gastrointestinal illness (AGI).
DALYs per 7-day backpacking trip	$DALY_{trip}$ (DALYs per trip)	$DALY_{trip} = P_{ill,trip} \cdot S \cdot DW$ (6.7) where $S =$ susceptible fraction of the population (100% for both *Campylobacter* and *Giardia)*	Haas et al.[361] *Campylobacter:* Bivins et al.[31] *Giardia*: Health Canada[370]
Difference in dose	ΔD_{trip} (CFU or oocysts per trip)	$\Delta D_{trip} = D_{trip,\ field\ LRV} - D_{trip,\ manuf\ LRV}$ (6.8)	Calculated by subtracting dose calculated using manufacturer-claimed LRVs from dose using field-obtained LRVs, such that a negative ΔD_{trip} indicates a higher dose under a manufacturer-claimed LRV.
Difference in P_{inf}	ΔP_{inf} (unitless)	$\Delta P_{inf} = P_{inf,\ field\ LRV} - P_{inf,\ manuf\ LRV}$ (6.9)	See above explanation for ΔD_{trip}.
Difference in $DALY_{trip}$	$\Delta DALY_{trip}$ (DALYs per trip)	$\Delta DALY_{trip} = DALY_{trip,\ field\ LRV} - DALY_{trip,\ manuf\ LRV}$ (6.10)	See above explanation for ΔD_{trip}.

6.5 Results

6.5.1 Field challenge tests

Of the 144 participants enrolled in the study, 3 withdrew without completing the interview due to reasons of time constraint or unexpected difficulty operating their POUWT device (Figure 6.3). There were 141 participants who completed the questionnaire (results of the questionnaire are outside the scope of the present publication), and 129 participants who completed the field challenge test. Of these, 25 participants were using POUWT devices for which LRV claims could not be found, for a total of 104 comparisons made between field-obtained and manufacturer-claimed LRVs, each for bacterial and protozoan pathogen categories.

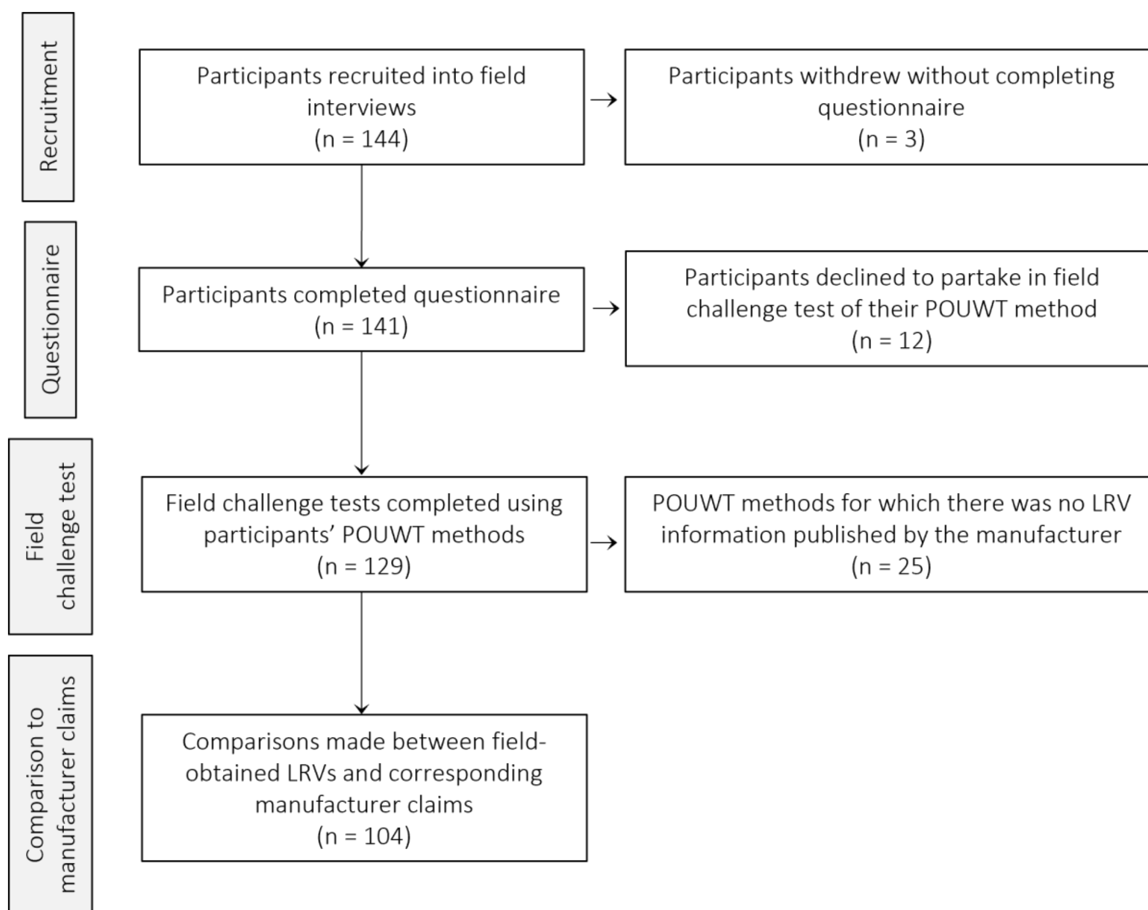

Figure 6.3: Selection criteria for inclusion of challenge test results in the QMRA model.

Field-obtained mean LRVs are presented in Table 6.2, compared with manufacturer-claimed LRVs. The most popular method of POUWT treatment was filtration, with 83 of 104 (79.80%) participants using a filter. Chemical disinfection was used by 16 of 104 (15.38%) of participants, either via liquid chlorine dioxide (ClO_2) drops or sodium dichloroisocyanurate (NaDCC) tablets. UV disinfection, of which there was only one brand studied (SteriPen), was used by 5 of 104 (4.81%) participants.

Regarding bacterial reduction, 101 (97.11%) of the 104 POUWT methods we examined performed worse in the field than claimed by manufacturers. Outcomes for protozoan (or *S. cerevisiae* surrogate) reduction differed from their bacterial counterparts: 42 of 104 (40.38%) POUWT devices performed worse in the field compared to published manufacturer claims, while 57 of 104 (54.81%) devices had higher performance in the field than reported by the manufacturer, and 5 of 104 (4.81%) performing identically to manufacturer claims.

*Table 6.2: Summary of field-obtained LRV results, with corresponding manufacturer-claimed LRVs for bacterial and protozoan pathogen categories. NA indicates not applicable; NS indicates not specified; * indicates LRV selected to compare against field-obtained LRVs. Summary values of manufacturer-claimed LRVs are weighted averages by the number of participants using each POUWT device.*

Treatment category	POUWT name (manufacturer)	Number of field challenge tests (% of total)	Mean field-obtained LRV (95% CI)	Manufacturer-claimed LRV (organism name or 95% CI as applicable)	Reference
Bacteria	All methods	104 (100%)	3.18 (2.97; 3.39)	6.11 (5.92; 6.31)	
Chemical disinfection	Aquatabs; NaDCC tablets (Medentech Ltd.)	11 (10.58%)	3.03 (2.30; 3.76)	6 (NS)	Mendentech Ltd.[371]
				6 (*E. coli*)	WHO[355]
	Pristine Water Purification System; ClO_2 drops (Pristine Canada)	5 (4.81%)	2.85 (2.45; 3.25)	6 (NS)	Pristine Canada[372]
	Overall chemical disinfection	16 (15.38%)	2.98 (2.47; 3.49)	6 (NA)	
Filtration	Grayl (Grayl USA)	6 (5.77%)	2.61 (1.54; 3.68)	6 (*E. coli, V. cholerae*, Salmonella)	Grayl USA[373]
	Base Camp Pro 10L (Katadyn Group)	1 (0.96%)	1.72 (NA)	6 (*K. terrigena*)	Katadyn Group[374]
	BeFree, 0.6 L or 1.0 L (Katadyn Group)	20 (19.23%)	3.29 (2.81; 3.78)	6 (*K. terrigena*)	Katadyn Group[375]
	Hiker or Hiker Pro (Katadyn Group)	4 (3.85%)	3.12 (1.97; 4.28)	6 (*K. terrigena*)	Katadyn Group[376]
	Katadyn Vario (Katadyn Group)	3 (2.88%)	3.32 (1.64; 4.99)	6 (*K. terrigena*)	Katadyn Group[377]
	Lifestraw Flex (Vestergaard)	1 (0.96%)	1.55 (NA)	8.66 (*E. coli*)	Nguyen et al.[378]
	Miniwell L630 (Miniwell Filters)	1 (0.96%)	4.38 (NA)	4 (NS)	Miniwell Filters[379]

	Autoflow gravity filter (Mountain Safety Research, MSR)	2 (1.92%)	3.83 (3.73; 3.92)	6 (NS)	MSR[380]
	Guardian (MSR)	1 (0.96%)	3.89 (NA)	6 (NS)	MSR[381]
	Hyperflow (MSR)	4 (3.85%)	3.03 (2.40; 3.65)	6 (NS)	MSR[382]
	Miniworks (MSR)	6 (5.77%)	2.96 (2.25; 3.68)	6 (NS)	MSR[383]
	Trailshot (MSR)	6 (5.77%)	2.97 (2.31; 3.63)	6 (NS)	MSR[384]
	GravityWorks (Platypus)	12 (11.53%)	3.88 (3.49; 4.27)	6 (*E. coli, V. cholerae*, Salmonella)	Platypus[385]
	Pristine Straw (Pristine Canada)	1 (0.96%)	4.83 (NA)	7 (NS)	Pristine Canada[372]
	Micro Squeeze (Sawyer Products)	1 (0.96%)	5.07 (NA)	7 (*E. coli, V. cholerae*, Salmonella)	Sawyer Products[386]
	MINI (Sawyer Products)	6 (5.77%)	3.93 (3.53; 4.33)	7 (*E. coli, V. cholerae*, Salmonella)	Sawyer Products [387]
				8.56* (*R. terrigena* ATCC 33628)	The Collaboratory[388]
	Squeeze (Sawyer Products)	6 (5.77%)	2.88 (2.35; 3.40)	7 (*E. coli, V. cholerae*, Salmonella)	Sawyer Products[389]
	Pro X Electric (Survivor Filter)	1 (0.96%)	3.13 (NA)	5 (*E. coli* and *S. aureus*)	Survivor Filter[390]
				6.95 (*E. coli* ATTC 11229)	Intertek[391]
				7.00* (*S. aureus* ATTC 6538)	
	Versa Flow (HydroBlu)	1 (0.96%)	2.82 (NA)	8 (*E. coli, V. cholerae*, Salmonella)	HydroBlu[392]
	Overall filters	83 (79.80%)	3.30 (3.09; 3.52)	6.32 (6.14; 6.50)	
UV disinfection	SteriPen (Katadyn Group)	5 (4.81%)	1.86 (0.64; 3.07)	3 (NS)	Katadyn Group[393]
Protozoa	All methods	104 (100%)	3.84 (3.59; 4.08)	3.69 (3.43; 3.95)	
Chemical disinfection	Aquatabs; NaDCC tablets (Medentech Ltd.)	11 (10.58%)	3.09 (2.44; 3.73)	3* (*G. lamblia*)	Mendentech Ltd.[371]
				0.2 (*C. parvum*)	WHO[355]

	Pristine Water Purification System; ClO_2 drops (Pristine Canada)	5 (4.81%)	1.79 (1.43; 2.16)	6 (*G. lamblia, C. parvum*)	Pristine Canada[372]
	Overall chemical disinfection	16 (15.38%)	2.68 (2.14; 3.22)	3.94 (3.23; 4.64)	
Filtration	Grayl (Grayl USA)	6 (5.77%)	3.26 (1.92; 4.60)	3 (*G. lamblia, C. parvum*)	Grayl USA[373]
	Base Camp Pro 10L (Katadyn Group)	1 (0.96%)	4.86 (NA)	3 (*G. lamblia, C. parvum*)	Katadyn Group[374]
	BeFree, 0.6 L or 1.0 L (Katadyn Group)	20 (19.23%)	4.17 (3.79; 4.56)	3 (*G. lamblia, C. parvum*)	Katadyn Group[375]
	Hiker or Hiker Pro (Katadyn Group)	4 (3.85%)	4.27 (2.83; 5.71)	3 (*G. lamblia, C. parvum*)	Katadyn Group[376]
	Katadyn Vario (Katadyn Group)	3 (2.88%)	2.86 (2.26; 3.46)	3 (*G. lamblia, C. parvum*)	Katadyn Group[377]
	Lifestraw Flex (Vestergaard)	1 (0.96%)	4.08 (NA)	5.2 (3 µm spheres)	Nguyen et al.[378]
	Miniwell L630 (Miniwell Filters)	1 (0.96%)	3.77 (NA)	4 (NS)	Miniwell Filters[379]
	Autoflow gravity filter (Mountain Safety Research, MSR)	2 (1.92%)	5.07 (3.56; 6.59)	3 (NS)	MSR[380]
	Guardian (MSR)	1 (0.96%)	3.70 (NA)	3 (NS)	MSR[381]
	Hyperflow (MSR)	4 (3.85%)	3.26 (2.24; 4.27)	3 (NS)	MSR[382]
	Miniworks (MSR)	6 (5.77%)	3.80 (2.91; 4.70)	3 (NS)	MSR[383]
	Trailshot (MSR)	6 (5.77%)	4.43 (3.69; 5.17)	3 (NS)	MSR[384]
	GravityWorks (Platypus)	12 (11.53%)	4.50 (3.73; 5.26)	3 (NS)	Platypus[385]
	Pristine Straw (Pristine Canada)	1 (0.96%)	5.39 (NA)	4 (NS)	Pristine Canada[372]

	Micro Squeeze (Sawyer Products)	1 (0.96%)	4.30 (NA)	6 (*G. lamblia, C. parvum*)	Sawyer Products[386]
	MINI (Sawyer Products)	6 (5.77%)	4.45 (3.52; 5.39)	6 (*G. lamblia, C. parvum*)	Sawyer Products [387]
				7.09* (*M. luteus* as surrogate for *G. lamblia*)	The Collaboratory[388]
				7.41 (*B. subtilis* as surrogate for *C. parvum*)	The Collaboratory[388]
	Squeeze (Sawyer Products)	6 (5.77%)	4.27 (3.48; 5.06)	6 (*G. lamblia, C. parvum*)	Sawyer Products[389]
	Pro X Electric (Survivor Filter)	1 (0.96%)	3.26 (NA)	5 (*G. lamblia, C. parvum*)	Survivor Filter[390]
	Versa Flow (HydroBlu)	1 (0.96%)	5.69 (NA)	8 (*G. lamblia, C. parvum*)	HydroBlu[392]
	Overall filters	83 (79.80%)	4.13 (3.88; 4.37)	3.68 (3.38; 3.98)	
UV disinfection	SteriPen (Katadyn Group)	5 (4.81%)	2.67 (1.49; 3.85)	3 (NS)	Katadyn Group[393]

6.5.2 QMRA

Comparisons between endpoints based on manufacturer-claimed and field-gathered LRV scenarios are shown for reference pathogens *C. jejuni* and *G. lamblia* on Figure 6.4 and Figure 6.5, respectively. Comparisons are shown for the three selected endpoints over a hypothetical 7-day backpacking trip: pathogen dose, probability of infection and expected DALYs (all via ingestion of drinking water); results are categorized by treatment type (i.e., chemical or UV disinfection, or filtration). Of note to Figure 6.5 (endpoints for *G. lamblia*), in cases where the endpoint data for each simulated "trip" vary widely due to widely varied input LRVs, a significant "jump" in the compiled CDF curve (i.e., the darker shaded curve) can be observed (e.g., Figure 6.5a, for chemical disinfection methods). A detailed breakdown of QMRA results, including 10th and 90th percentile data, medians and means, can be found in the supplementary information (SI, Table S6.4).

Generally, for the *C. jejuni* reference pathogen (bacterial surrogate), health risks are lower when QMRA is based on manufacturer-claimed LRVs, compared to the parallel scenario using field-obtained LRVs (Figure 6.4). This owes to the fact that field-obtained LRVs are nearly all lower than the corresponding manufacturer claims (Table 6.2) for the bacterial pathogen class. Comparative results for the *G. lamblia* reference pathogen are much more mixed (Figure 6.5), as many devices had higher performance in the field than reported by the manufacturer for the protozoan pathogen class (Table 6.2).

Health risks based on *C. jejuni* are several orders of magnitude higher than those from *G. lamblia* (Table S6.4), possibly due to the lower raw water concentration and lower average prevalence (Table 6.1). A subtractive comparison of health endpoints based on manufacturer-claimed versus field-gathered LRVs can be found in the supplementary information (Figure S6.6 and Figure S6.7).

The sensitivity of model endpoints was consistent for both reference pathogens for each respective model input. By a wide margin, the model was most sensitive to LRV as an input variable for both reference pathogens (Table 6.3), with high correlation coefficients ranging from -0.953 to -0.932. LRV sensitivity was followed by raw water pathogen concentration, total volume of water ingested during the 7-day trip, and the probability of illness given infection (Table 6.3).

Table 6.3: Summary of the pairwise sensitivity analysis.

Microbe	End point	Input variable (units)	Spearman correlation, ρ
C. jejuni	Dose	LRV (unitless)	-0.936
		Total drinking water consumed (L)	0.019
		Raw water pathogen concentration, C_{raw} (CFU/L)	0.316
	P_{inf}	LRV (unitless)	-0.937
		Total drinking water consumed (L)	0.020
		Raw water pathogen concentration, C_{raw} (CFU/L)	0.314
	DALYs	LRV (unitless)	-0.932
		Total drinking water consumed (L)	0.019
		Raw water pathogen concentration, C_{raw} (CFU/L)	0.305
		Probability of illness given infection, $P_{ill\mid inf}$ (unitless)	0.117
G. lamblia	Dose	LRV (unitless)	-0.953
		Total drinking water consumed (L)	0.057
		Raw water pathogen concentration, C_{raw} (CFU/L)	0.210
	P_{inf}	LRV (unitless)	-0.953
		Total drinking water consumed (L)	0.057
		Raw water pathogen concentration, C_{raw} (CFU/L)	0.210
	DALYs	LRV (unitless)	-0.943
		Total drinking water consumed (L)	0.043
		Raw water pathogen concentration, C_{raw} (CFU/L)	0.156
		Probability of illness given infection, $P_{ill\mid inf}$ (unitless)	0.196

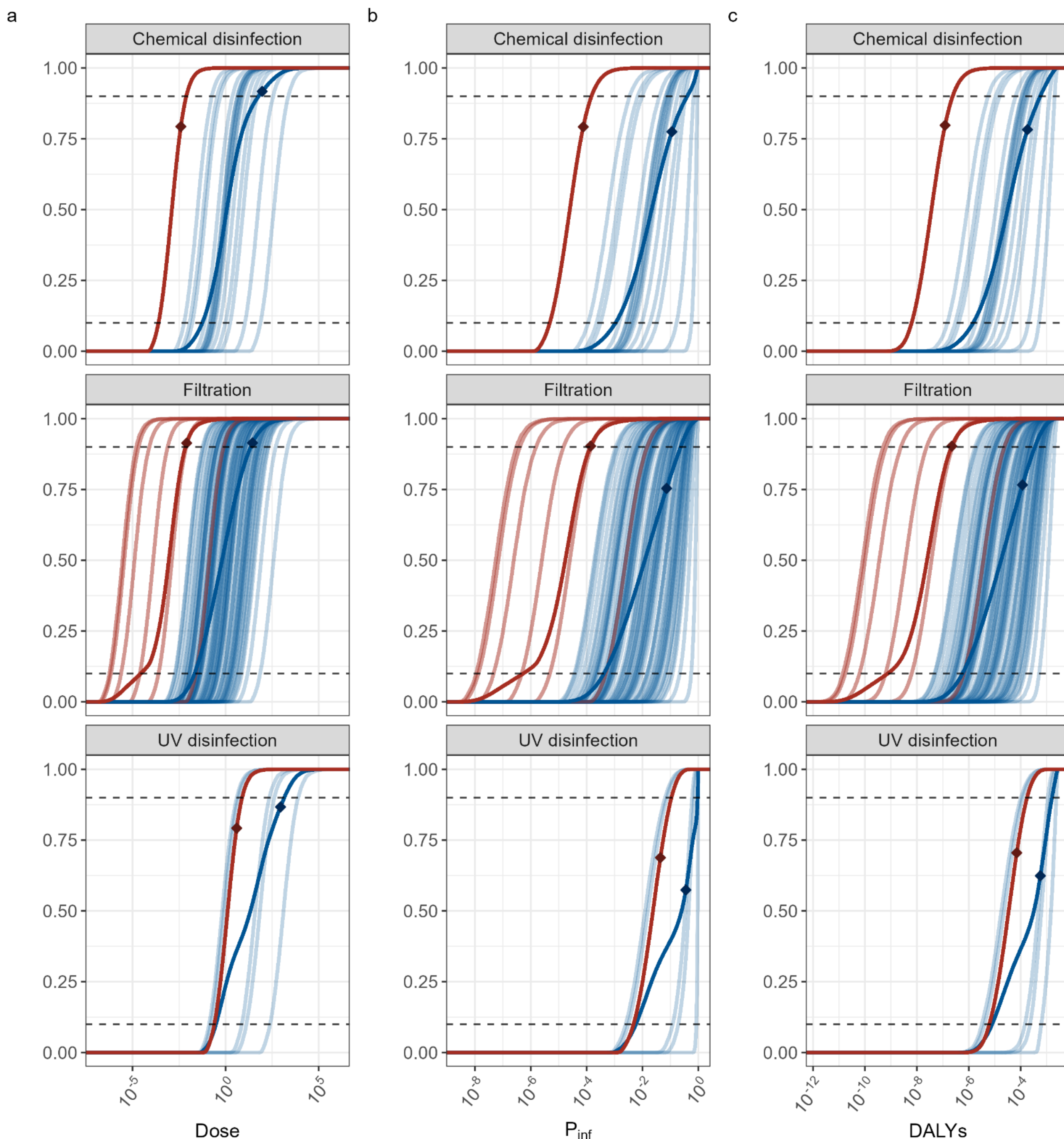

Figure 6.4: Cumulative distribution function (CDF) of QMRA endpoints comparing scenarios based on field-obtained (▬) and manufacturer-claimed (▬) LRVs. The y axis refers to the probability that a result will have less than or equal to the corresponding x-axis value. Lightly shaded lines represent QMRA simulations based on a single point-value input LRV. Darker red and blue lines represent compiled results for each treatment category. Diamond shapes represent the mean endpoint values for compiled results. Endpoints examined are a) dose of C. jeujeni *ingested via drinking water per 7-day backpacking trip; b) probability of infecction with* C. jeujeni *via drinking water per 7-day backpacking trip; and c) DALYs arising from ingestion of* C. jeujeni *via drinking water per 7-day backpacking trip.*

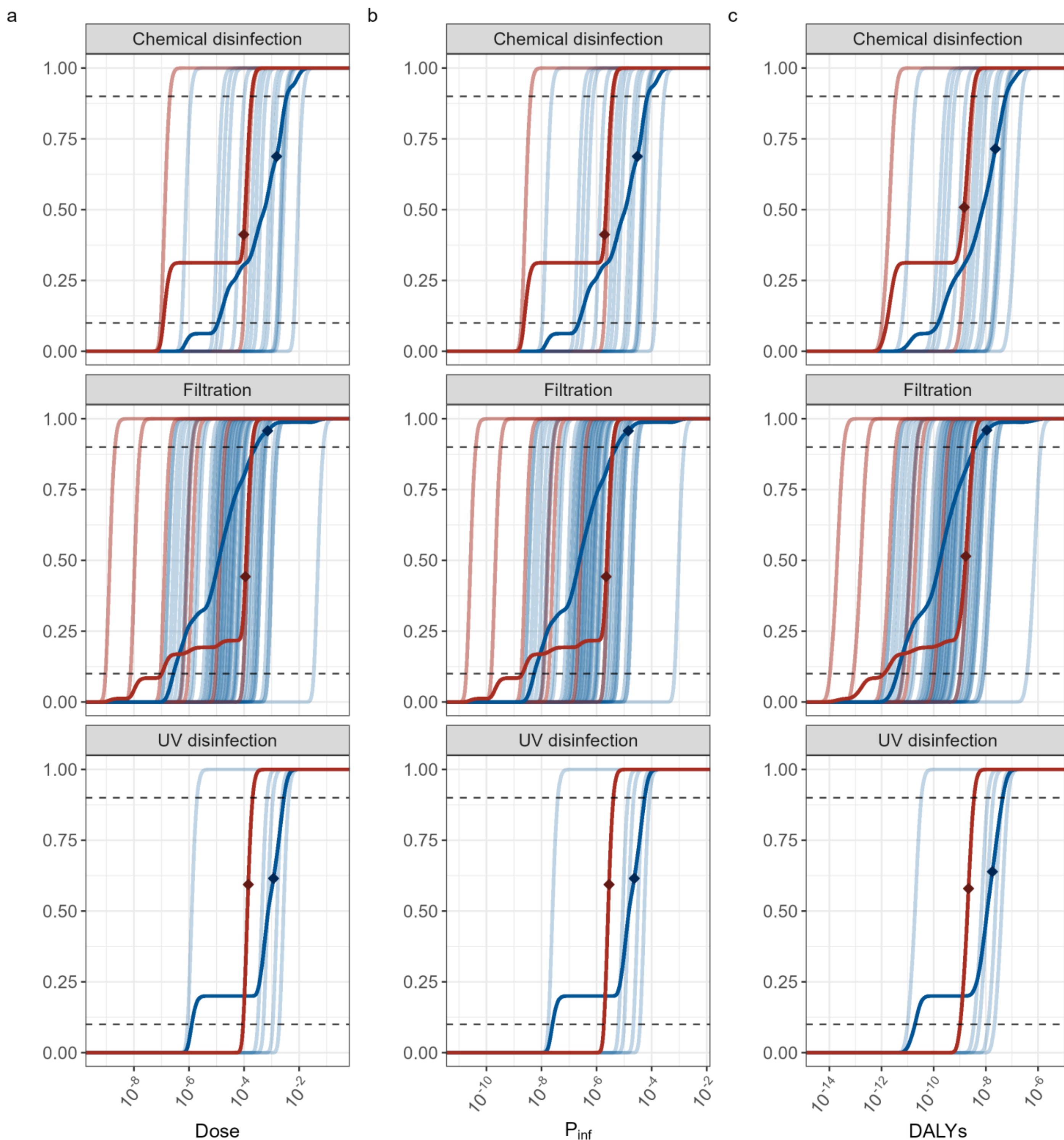

Figure 6.5: CDF of QMRA endpoints comparing scenarios based on field-obtained (—) and manufacturer-claimed (—) LRVs. The y axis refers to the probability that a result will have less than or equal to the corresponding x-axis value. Lightly shaded lines represent QMRA simulations based on a single point-value input LRV. Darker red and blue lines represent compiled results for each treatment category. Diamond shapes represent the mean endpoint values for compiled results. Dashed lines represent 10% and 90% percentiles for visual aid. Endpoints examined are a) dose of G. lamblia *ingested via drinking water per 7-day backpacking trip; b) probability of infecction with* G. lamblia *via drinking water per 7-day backpacking trip; and c) DALYs arising from ingestion of* G. lamblia *via drinking water per 7-day backpacking trip.*

6.6 Discussion

6.6.1 Field challenge tests

Laboratory-obtained (or manufacturer-claimed) LRVs reflect the potential performance of POUWT methods under idealized conditions – what we can think of as the "optimized" performance[20], as well as possible water quality impacts. Field-based challenge testing can provide a comparative LRV that accounts for water quality, user behaviours, and general context.

In general, field-based LRVs are expected to be lower than their laboratory-obtained counterparts[20], and we did observe this with respect to the bacterial reduction from our field challenge testing compared to respective manufacturer claims (Table 6.2). However, the same was not true with respect to protozoan (or *S. cerevisiae* surrogate) reductions, with many devices showing better performance *in situ* compared to manufacturer claims (Table 6.2). This may be because manufacturer claims regarding protozoan reduction were relatively low compared to bacterial LRV claims, particularly regarding filtration technologies (Table 6.2). This contradicts what might be expected, given that manufacturers are likely incentivised to make high LRV claims for marketing purposes. The average protozoan LRV claimed by filter manufacturers was 3.68 (95% CI 3.38; 3.98), compared to 6.32 (95% CI 6.14; 6.50) for bacteria. In theory, membrane filtration technologies, which operate generally on size exclusion (Section 1.3.2), ought to be more efficacious at removing larger protozoans than smaller bacteria pathogen classes[51]. This was seen in the field challenge test data; for filters the average bacterial reduction was 3.30 $\log_{10}$ (95% CI 3.09; 3.52) while protozoan surrogate *S. cerevisiae* had an average LRV of 4.13 (95% CI 3.88; 4.37). Therefore, it seems possible that manufacturer-claimed LRVs for protozoan filtration are under-estimated, while bacterial LRVs have been over-estimated. The reasons for this are unknown, but could be due to challenges associated with working with protozoans, which can time-consuming, labour-intensive and technically complex[250,251,256]. It is possible that due to analytical challenges, a lower spike organism count was used for some protozoan challenge tests, resulting in relatively low but censored manufacturer claims, which could be higher if a higher spike was used. However, no manufacturers mentioned censorship in their LRV estimates for protozoan pathogens, nor reasoning for lower claims compared to bacterial reductions.

Identification of the highest-LRV water treatment technique under real-use conditions is one of the potential applications of field challenge testing[20]. Anecdotally, while conducting participant surveys, an extremely common question from participants was *"which device is the best for me to use?"*. We found that for bacterial reductions (i.e., probiotic *E. coli*), chemical disinfection and filtration methods performed comparably under field conditions, while UV disinfection performed more poorly (Table 6.2) – although this may be anomalous due to small sample size (n = 5 for UV disinfection). For protozoan reductions (i.e., *S. cerevisiae*) under field conditions, filters performed the best (an expected result considering the size exclusion treatment mechanism), followed by chemical and UV disinfection, which performed comparably (Table 6.2). The most protective choice of treatment type would be guided by QMRA; discussed in Section 6.6.2.

6.6.2 QMRA

General model characteristics

Modelled risks arising from ingestion of *C. jejuni* were substantially higher than those from *G. lamblia*, generally by several orders of magnitude (Figure 6.4, Figure 6.5, Figure S6.6, Figure S6.7 and Table S6.4). In many cases, the lowest 10th percentile endpoint from *C. jejuni* was higher than the highest 90th percentile endpoint from *G. lamblia*, given the same treatment category and context (Table S6.4). One plausible explanation for the differing health risks could be differing raw water concentrations of each reference pathogen; median input concentrations of *C. jejuni* were orders of magnitude higher than that of *G. lamblia* (Table 6.1), leading to order of magnitude differences in risk estimates (Table S6.4). This explanation is borne by examining dose estimates arising from field-based LRVs, which in many cases were comparable for bacterial and protozoan surrogates (Table 6.2). Given similar LRVs (i.e., in the field), dose estimates were orders of magnitude lower for *G. lamblia* than for *C. jejuni* reference pathogens (Table S6.4). This is supported by the sensitivity analysis; model parameters are the most sensitive to input LRVs, but raw water pathogen concentration comes second (Table 6.3); for similar LRVs, health risks will be driven by raw water concentration.

Given that modelled risks arising from the ingestion of *C. jejuni* were substantially higher than those arising from *G. lamblia* (Table S6.4), the choice of treatment type to maximise health protection would be decided by *C. jejuni*-based risks. Based on field-obtained LRVs, filtration and chemical disinfection POUWT methods give the highest health protection

against *C. jejuni*, with filtration providing slightly more protection in most cases (Table S6.4). However, health protection is only maximized if POUWT methods are operated consistently and correctly[31–34,80], with appropriate hygiene and water storage procedures[15,78]. A more protective approach is to use multiple treatment steps so that the gaps in one method are filled by the next (e.g., viruses can generally pass through filters but are inactivated by chemical disinfection)[19], although we did not examine this approach.

The underlying justification for the current work was that POUWT methods perform more poorly under field conditions than in the laboratory, leading to potential overestimation of health gains resulting from treatment[20]. However, we only partially observed the "laboratory versus field" health discrepancy in our work. Health risks stemming from *C. jejuni* were higher based on field-gathered LRVs than for corresponding manufacturer-claimed LRVs, by an order of magnitude in most cases (Figure 6.4, Figure S6.6 and Table S6.4). However, for the *G. lamblia* reference pathogen, we observed comparable health risks between field-gathered and manufacturer-claimed LRV scenarios (Figure 6.5, Figure S6.7 and Table S6.4). The difference (or lack thereof) in health risk estimates can be attributed to the difference in LRVs, given model construction (i.e., modelling two parallel backpacking trips with only the LRV varied between them; Figure 6.2 and Table 6.1). The similarity in computed health risks for *G. lamblia* brings up a previously unexplored justification for using *in situ* POUWT evaluations (i.e., field challenge testing): if *in situ* POUWT performance is comparable or higher than laboratory performance (as was seen in our study for *G. lamblia*), this is also valuable information. Technologies that are comparably or more protective in practice than under laboratory conditions are as important to identify as underperforming technologies, due to possible unexplored health benefits.

Model endpoints were found to be highly sensitive to input LRV (Table 6.3), which is consistent with published findings that treatment efficacy was frequently reported to be of high or highest influence on health risk estimates[128]. However, reductions in health risks (i.e., dose, P_{inf} and DALY endpoints) followed "the law of diminishing returns" with respect to LRV (Figure S6.8). That is, steadily declining health risks are observed up for LRVs of up to approximately 5 and 2 for *C. jejuni* and *G. lamblia*, respectively; then, for higher LRVs, very slight additional health gains were observed. The "inflection point" – the LRV at which minimal further health gains are observed – is intuitively a function of the raw water concentration. If raw water pathogen concentrations are low, as for *G. lamblia*, then

a lower LRV will be required to reduce pathogens to non-detectible levels in drinking water, beyond which further treatment produces negligible results in terms of health protection. For *C. jejuni*, having a generally higher raw water concentration, a higher LRV is required before drinking water reaches non-detectible pathogen concentrations.

Therefore, the focus on relatively high LRVs as claimed by POUWT manufacturers may be unnecessary for relatively low background microbe levels (e.g., in backcountry areas where water sources are minimally impacted by human activities). Indeed, highly efficacious water treatment devices may be more difficult to use than a less efficacious device, potentially leading to lower compliance and higher overall exposure than if the user-friendly but less efficient device is used[33]. Other work has also proven that POUWT adoption can be more important than achievement of high LRVs in protecting against waterborne disease[31,32,34,107], although we did not incorporate adoption into our model. Our model reflects 100% adoption of POUWT methods, although our survey data (unpublished) indicates that some survey participants did drink untreated water on occasion. Therefore, the potential health benefits conferred by POUWT methods while backpacking could be lower than simulated in this study.

Comparison of model with health-based guidelines and epidemiologic studies of backpackers

Epidemiologic studies estimate the prevalence of non-attributable diarrhea (i.e., not necessarily from drinking water contaminated with either *C. jejuni* or *G. lamblia* reference pathogens) vary widely depending on context. A high prevalence has been observed on the Appalachian Trail with estimates of 56% and 63% by Boulware et al.[394] and Crouse [47], respectively. In the summer of 2022, a norovirus-associated outbreak of acute gastroenteritis occurred in the backcountry of the Grand Canyon National Park, USA, with attack rates of up to 83%[395]. Despite high prevalence reports in specific geographical and/or temporal contexts, the overall prevalence of diarrhea in backpackers has been estimated as 3-5% across the United States[48]. Water treatment, in conjunction with good hygiene, is the best way to avert the risk of diarrhoeal illness[44,48]; a study of hikers from the Appalachian Trail found that hikers who drank untreated water from streams or ponds more than several times per week were more likely to have diarrhoea (OR = 7.7; 95% CI: 2.7 to 23), at an increased rate of 1.6 - 2.4 days per month[394].

For comparison, our model produced a probability of infection with *C. jejuni* of $2.13 \cdot 10^{-2}$, $1.20 \cdot 10^{-2}$, and $2.51 \cdot 10^{-1}$ for chemical disinfection, filtration and UV disinfection respectively (Table S6.4). In our model we assumed that persons infected with *C. jejuni* have a 10% to 60% chance of developing AGI symptoms (Table 6.1). Therefore, under our model hikers have a lower probability of diarrheal illness than reported prevalence in the literature for chemical disinfection and filtration treatment methods, but comparable (i.e., similar order of magnitude) to 3-5% prevalence estimates by Zell[48] if using UV disinfection methods, which had a lower observed LRVs from field challenge testing (Table 6.2). As noted above, literature reports of diarrheal prevalence do not differentiate the pathogenic cause or transmission route, whereas our model estimates are based on specific reference pathogens and drinking water transmission route.

WHO health-based guidelines adopt 10^{-6} DALY/person per year as a health target for drinking water[396], translating to 1.92 10^{-8} DALY/person per week. Our lowest 10th percentile DALY estimates were all higher than the WHO[396] guideline, using field-obtained LRVs with *C. jejuni* as reference pathogen (Table S6.4). With *G. lamblia* as the reference pathogen, calculated DALYs from filtration treatment methods were below the WHO guidelines at the 90th percentile (Table S6.4), while UV and chemical disinfection methods were approximately equal to the WHO guidelines at the 90th percentile (Table S6.4). Therefore, most health risks were generally lower than WHO guidelines with *G. lamblia* as the pathogen; field-based *C. jejuni* risk estimates were uniformly higher than WHO guidelines. However, given that backpacking is a recreative activity in which risk is voluntarily undertaken by healthy individuals, the WHO guidelines may not be fully applicable, as they are intended for long-term drinking water sources. Although not directly applicable to drinking water, the USEPA health guidelines for recreational water quality (i.e., for swimming) state 3.2% to 3.6% probability of illness as an acceptable risk[397], higher than our risk estimates for *C. jejuni* as a reference pathogen, and comparable to the 3-5% illness prevalence estimated for backcountry hikers in the United States[48]. Therefore, an elevated risk of illness may be tolerable to those hiking in the backcountry.

Model applications and broader implications

Our general conclusions are limited by the study context: relatively healthy individuals voluntarily undertaking safety and health risks for a limited time; relatively high-quality source water available in the backcountry. However, POUWT methods are widely used,

with over 1 billion people globally treating their drinking water at home as an effective barrier against waterborne illness[43].

QMRA models have used laboratory-based LRVs as model inputs to estimate health gains conferred by POUWT methods[30,105–107]. Potential limitations of using laboratory-based LRV estimates are not typically examined, despite evidence that POUWT methods do not perform optimally under real use conditions[20]. Use of an accurate $\log_{10}$ reduction has a high impact on QMRA analyses, and consequently, the recommendations stemming from those analyses[398], and therefore site-specific data is preferred to decrease the level of uncertainty[123]. Although adoption was not considered in our model, and it accepted that this parameter is essential to QMRA modelling when estimating health gains by POUWT[31,32,34,121]. We posit that use of field-gathered LRVs could and should be incorporated into QMRA models in the same manner.

The framework we present here can readily be used to evaluate POUWT methods *in situ* and predict health gains, both independently and relative to laboratory-based LRVs. Field-based evaluation methods reflect the total context in which POUWT methods are used and can be utilized to tease out individual factors that could compromise (or strengthen) performance. Laboratory-based evaluations are a key step to screen potential technologies, however *in situ* evaluations could be used to quantify myriad contextual impacts for design and improvement of POUWT methods. Field data could also inform instructional campaigns for best practices regarding POUWT methods.

An eventual possibility could be to conceptualize field performance as a dependent variable: a function of potential optimized performance (i.e., the laboratory-based LRV), plus or minus multiple regression coefficients that account for factors such as specific user behaviours, water quality, and other contextual influences. Predicting a given field LRV as a function of laboratory-based LRV and other contextual factors would be a powerful tool, and could aid in appropriate technology selection for a given context. A conceptually similar regression model was successfully used by Ishaq et al.[399] to predict pathogen concentration in (and resulting health risks from exposure to) stormwater, as a function of contextual factors of seasonality, temperature, geographic location, stormwater receiving body and management strategy.

There have been recent developments towards testing and implementing a sanitary inspection/survey for POUWT methods[400,401], based on established sanitary survey

methods for drinking water sources[402] and intended to augment established techniques to evaluate household water treatment and safe storage approaches[116,120]. Field challenge testing and subsequent QMRA could aid in validation of specific sanitary inspection items as an evidence-based way to support the inclusion or exclusion of specific criterion into the inspection.

6.6.3 Limitations

Field challenge testing

Limitations and corresponding implications of the field challenge test itself, mainly related to physiological state of spike microbes dissolved from powder and field quality control limitations, have been discussed elsewhere[20]. In addition, although work has been done to validate EcN and S. cerevisiae as surrogates for bacterial and protozoan pathogens, respectively (see Chapter 3 through Chapter 5), there is no such thing as a perfect surrogate[111] and all surrogate selections involve decision trade-offs[111,226,227]. The data provided by using EcN and S. cerevisiae is valuable and overall representative of a given POUWT performance with respect to a pathogen class, however the LRVs gathered here may not be exactly the same as if devices were tested using pathogenic bacterial or protozoan spike microorganisms.

Beyond those protocol-specific limitations, the device-specific field challenge test results (Table 6.2) generally have a small sample size; in some cases, only one challenge test was conducted for a given device brand. At the time of the survey, data was collected regarding the age of the given POUWT device (e.g., date of purchase of the participant's chlorine tablets), participant's experience regarding ease of use, visual inspection of the POUWT device (e.g., visual observation of cracks in a filter). However, due to the low sample size within each device brand tested, we were not able to assess the relationship between these factors and the device LRV (topic of an upcoming publication).

The objective of this work was not to conclusively establish field-based estimates for any specific device, but rather examine the potential impacts of using laboratory-based or manufacturer-claimed LRVs to health risk assessments, so the small sample size did not hinder our objectives. However, our field-based LRVs should not be taken as comprehensive estimates of field performance of a given device due to the small sample size and context-specific factors such as water quality. Generalized field LRV estimates

would need to be verified by targeted research having a larger sample size under a greater variety of contexts.

It is also important to note that in many cases the manufacturer claims referenced for comparison in this study are just that: claims made by the manufacturer. None of the claims were supported by peer-reviewed publications, and manufacturer claims were supported by third party testing for only 3 out of the 22 (13.6%) technology brands studied. Aquatabs were tested under the WHO Scheme[355], the Pro X Electric filter (Survivor Filter) was tested by Intertek[391], and the MINI filter (Sawyer Products) was tested by The Collaboratory[388]. The remaining claims were not supported by third-party testing, and in many cases, no protocol and/or data were referenced. However, manufacturer claims that were supported by third-party testing generally did not align with corresponding field LRVs any better than claims that were not supported by third-party testing (Table 6.2). In some cases advertised LRVs did not refer to typical spike organisms or reference pathogens, (e.g., use of *K. terrigena* instead of *E. coli*) and so differences in performance may also be attributed to differing organism responses to treatment mechanisms. However, manufacturer claims are still instructive, as consumers potentially rely on them to make purchasing decisions and compare devices.

QMRA

Assumptions and limitations are inherent to QMRA modelling and results should be interpreted considering these. The spike organisms used for field challenge testing in our work were EcN (a probiotic supplement) and *S. cerevisiae* (baker's yeast) as surrogates for bacterial and protozoan pathogens, respectively. *S. cereviaiae* has been validated against protozoan pathogens with respect to filtration[37,38] and against *G. lamblia* with respect to UV disinfection (Chapter 5), while EcN has been validated against widely-used laboratory strain *E. coli* K-12 for filtration, chemical disinfection and UV disinfection (Chapter 3 and Chapter 4). We assume here that devices tested with *C. jejuni* would perform the same as they would spiked with EcN; challenge testing is often carried out using *E. coli* and subsequently contextualized via the WHO star ratings, which were designed based on *C. jejuni* as the bacterial reference pathogen[19]. However, EcN has not been validated as a surrogate for *C. jejuni.*

Raw water pathogen prevalence and concentrations were taken from water quality monitoring studies in similar contexts[362–364], as we did not collect reference pathogen water

quality data from our study site due to time and resource constraints. It is possible that pathogen concentrations and/or prevalence differed in our case, however as our model is intended to be comparative the impacts on our conclusions would be minimal. Raw water quality is likely to be variable between settings[31] and is a key input to QMRA[398] and therefore context-specific inputs would be required if applying our model framework to other locations. Although our dose-response models are generally widely used[134], they are generated using a small data pool and correspondingly high potential error[403], and originate from healthy adults in high income countries, limiting potential extrapolation to other contexts[404].

We used point-value LRVs (either field-obtained or corresponding manufacturer claims) as inputs to our QMRA model under the assumption that a given POUWT device delivers constant performance throughout a 7-day backpacking trip. However, it could be possible for POUWT performance to vary due to potential reasons such as user fatigue or variations in raw water quality during the trip. The field-based LRVs were obtained via field challenge testing as a part of a survey administered by the authors, which was by nature unblinded. The observed LRVs are therefore open to observational bias: participants could have been on their "best behaviour" regarding their water treatment activities, a phenomenon which has been noted in health outcome studies of POUWT methods in other contexts[61,405,406]. Bias aside, a 7-day backpacking trip is a relatively short amount of time to be treating water for drinking in comparison to contexts where POUWT devices must be used indefinitely in the household to protect against illness. In such scenarios, a decline in POUWT effectiveness and adoption over time is well documented in the literature[24,81,100,102]. It is therefore possible that our field-based LRVs have been overestimated and corresponding health risks underestimated; however, even with the possibility of observational bias, field challenge testing would be more reflective of end user conditions than laboratory LRV estimates. Estimates of drinking water consumption were also based on the authors' field survey data, and therefore is subject to recall bias or inaccurate recollections of daily water consumption[407].

As there are currently no food-safe viral surrogates available at the time of this study, we were not able to include viruses in our field challenge test evaluations, and viral LRVs have likewise been excluded from our QMRA model. This could have impacts for the estimated health protection conferred by filtration treatment methods in particular, whose main treatment mechanism is size exclusion, making it difficult to remove viruses, which

are order(s) of magnitude smaller than bacterial and protozoan pathogens[51]. The WHO[19] estimates a maximum LRV of 4.0 of viruses via ceramic filtration; this is similar to manufacturer claims of 4-5 LRV for some filtration methods[373,390]. However, the baseline expected LRV of viruses via ceramic filtration in actual field practice is 1.0, in the one-star performance category[19]. Before the WHO baseline estimate can be verified, and the corresponding health impacts quantified, a food-safe viral surrogate must be identified and validated[20].

Although the focus of this study is on health risks and exposure to reference pathogens *C. jejuni and G. lamblia* via ingestion of drinking water, exposure and illness is also possible resulting from other waterborne pathogens while backpacking. Other routes such as ingestion via cooking or hand-to-mouth transmission are also possible[408]; indeed, we collected data indicating a relatively high presence of faecal indicator bacteria on participants' hands (topic of future publication).

Despite the limitations discussed here, we believe that our QMRA is useful and informative as conducted. Given that the model is intended to facilitate internal comparisons, there is inherent internal validity to the analysis as most assumptions apply equally to both field-based and manufacturer-claimed LRV scenarios.

6.7 Conclusion

The overall aim of this work was to provide a framework that could be used to gather and contextualize field-relevant LRVs of POUWT methods. The method of using food-safe bacterial and protozoan surrogates to carry out field challenge testing was successfully implemented in this study. Using our field-based LRV results, we sought to compare the health impacts of using laboratory-based vs field-obtained LRV data on health risk assessments via a QMRA analysis.

For the bacterial pathogen class, calculated health risks based on field-obtained (i.e., by field challenge testing) were substantially higher compared to corresponding manufacturer-claimed LRVs, which were higher than seen in the field. For the protozoan pathogen class, calculated health risks were similar, due to homogeneity between field-obtained and manufacturer-claimed LRVs. However, manufacturer-claimed LRVs were supported by third party testing for only 3 out of the 22 (13.62%) brands studied and are therefore potentially speculative.

The QMRA analysis we conducted can be more broadly applied to contextualize findings from future field challenge testing campaigns. This has numerous implications including validation of POUWT sanitary inspection criteria, quantifying health impacts of contextual factors, or to inform technology selection. However, our analysis here is conducted in the context of backcountry camping conditions, and model inputs would need context-specific adjustment prior to additional applications.

6.8 Author contributions

Conceptualization: CZ and CCD; methodology: CZ, AB and CCD; field data collection: CZ and AG; statistical analysis: CZ; QMRA modelling: CZ and AB; figure preparation: CZ; writing – original draft: CZ; writing – review and editing: CZ, AB and CCD; supervision: AB and CCD.

6.9 Supplementary information

Table S6.4: Summary of QMRA endpoints based on manufacturer claimed LRVs and field-gathered LRVs, for a) C. jejuni *and b)* G. lamblia *reference pathogens. Negative values indicate that health risks are higher under manufacturer-claimed LRVs than corresponding field-obtained values.*

a. *C. jejuni*

LRV source	Treatment category	Dose (CFU ingested via drinking water)				Probability of infection (via drinking water)				DALYs (attributed to drinking water)			
		10th percentile	90th percentile	Median	Mean	10th percentile	90th percentile	Median	Mean	10th percentile	90th percentile	Median	Mean
Manufacturer-claimed	Chemical disinfection	$2.50 \cdot 10^{-4}$	$7.67 \cdot 10^{-3}$	$1.29 \cdot 10^{-3}$	$3.96 \cdot 10^{-3}$	$4.80 \cdot 10^{-6}$	$1.48 \cdot 10^{-4}$	$2.48 \cdot 10^{-5}$	$7.58 \cdot 10^{-5}$	$1.91 \cdot 10^{-8}$	$6.12 \cdot 10^{-4}$	$1.26 \cdot 10^{-4}$	$2.23 \cdot 10^{-4}$
	Filtration	$2.81 \cdot 10^{-5}$	$6.98 \cdot 10^{-3}$	$8.91 \cdot 10^{-4}$	$7.76 \cdot 10^{-3}$	$5.40 \cdot 10^{-7}$	$1.34 \cdot 10^{-4}$	$1.72 \cdot 10^{-5}$	$1.36 \cdot 10^{-4}$	$2.76 \cdot 10^{-9}$	$2.51 \cdot 10^{-7}$	$2.80 \cdot 10^{-8}$	$2.50 \cdot 10^{-7}$
	UV disinfection	$3.08 \cdot 10^{-1}$	$7.73 \cdot 10^{0}$	$1.29 \cdot 10^{0}$	$4.00 \cdot 10^{0}$	$4.80 \cdot 10^{-3}$	$1.08 \cdot 10^{-1}$	$2.37 \cdot 10^{-2}$	$4.36 \cdot 10^{-2}$	$6.13 \cdot 10^{-6}$	$1.76 \cdot 10^{-4}$	$3.43 \cdot 10^{-5}$	$7.06 \cdot 10^{-5}$
Field-obtained	Chemical disinfection	$5.56 \cdot 10^{-2}$	$5.81 \cdot 10^{1}$	$1.16 \cdot 10^{0}$	$8.91 \cdot 10^{1}$	$1.07 \cdot 10^{-3}$	$3.95 \cdot 10^{-1}$	$2.13 \cdot 10^{-2}$	$1.12 \cdot 10^{-1}$	$1.46 \cdot 10^{-6}$	$5.64 \cdot 10^{-4}$	$3.10 \cdot 10^{-5}$	$1.81 \cdot 10^{-4}$
	Filtration	$2.16 \cdot 10^{-2}$	$2.22 \cdot 10^{1}$	$6.41 \cdot 10^{-1}$	$2.70 \cdot 10^{1}$	$4.15 \cdot 10^{-4}$	$2.31 \cdot 10^{-1}$	$1.20 \cdot 10^{-2}$	$7.26 \cdot 10^{-2}$	$5.75 \cdot 10^{-7}$	$3.51 \cdot 10^{-4}$	$1.74 \cdot 10^{-5}$	$1.17 \cdot 10^{-4}$
	UV disinfection	$2.51 \cdot 10^{-1}$	$7.71 \cdot 10^{0}$	$1.30 \cdot 10^{0}$	$3.92 \cdot 10^{0}$	$5.83 \cdot 10^{-3}$	$9.39 \cdot 10^{-1}$	$2.51 \cdot 10^{-1}$	$3.55 \cdot 10^{-1}$	$7.89 \cdot 10^{-6}$	$1.60 \cdot 10^{-3}$	$3.21 \cdot 10^{-4}$	$5.72 \cdot 10^{-4}$
Difference between manufacturer-claimed and field-obtained LRV scenarios	Chemical disinfection	$5.43 \cdot 10^{-2}$	$5.81 \cdot 10^{1}$	$1.16 \cdot 10^{0}$	$8.91 \cdot 10^{1}$	$1.04 \cdot 10^{-3}$	$3.95 \cdot 10^{-1}$	$2.13 \cdot 10^{-2}$	$1.12 \cdot 10^{-1}$	$1.43 \cdot 10^{-6}$	$5.58 \cdot 10^{-4}$	$3.10 \cdot 10^{-5}$	$1.80 \cdot 10^{-4}$
	Filtration	$1.84 \cdot 10^{-2}$	$2.22 \cdot 10^{1}$	$6.36 \cdot 10^{-1}$	$2.70 \cdot 10^{0}$	$3.53 \cdot 10^{-4}$	$2.31 \cdot 10^{-1}$	$1.19 \cdot 10^{-2}$	$7.24 \cdot 10^{-2}$	$4.88 \cdot 10^{-7}$	$3.50 \cdot 10^{-4}$	$1.73 \cdot 10^{-5}$	$1.16 \cdot 10^{-6}$
	UV disinfection	$-1.33 \cdot 10^{0}$	$1.40 \cdot 10^{3}$	$2.03 \cdot 10^{1}$	$8.18 \cdot 10^{2}$	$-2.06 \cdot 10^{-2}$	$8.94 \cdot 10^{-1}$	$2.37 \cdot 10^{-1}$	$3.12 \cdot 10^{-1}$	$-3.12 \cdot 10^{-5}$	$1.46 \cdot 10^{-3}$	$2.91 \cdot 10^{-4}$	$5.00 \cdot 10^{-4}$

b. *G. lamblia*

LRV source	Treatment category	Dose (CFU ingested via drinking water)				Probability of infection (via drinking water)				DALYs (attributed to drinking water)			
		10th percentile	90th percentile	Median	Mean	10th percentile	90th percentile	Median	Mean	10th percentile	90th percentile	Median	Mean
Manufacturer-claimed	Chemical disinfection	$1.14 \cdot 10^{-7}$	$1.88 \cdot 10^{-4}$	$1.10 \cdot 10^{-4}$	$9.76 \cdot 10^{-5}$	$2.28 \cdot 10^{-9}$	$3.75 \cdot 10^{-6}$	$2.19 \cdot 10^{-6}$	$1.94 \cdot 10^{-6}$	$1.57 \cdot 10^{-12}$	$3.18 \cdot 10^{-9}$	$1.46 \cdot 10^{-9}$	$1.49 \cdot 10^{-9}$
	Filtration	$1.02 \cdot 10^{-7}$	$1.94 \cdot 10^{-4}$	$1.18 \cdot 10^{-4}$	$1.12 \cdot 10^{-4}$	$2.03 \cdot 10^{-9}$	$3.86 \cdot 10^{-6}$	$2.35 \cdot 10^{-6}$	$2.22 \cdot 10^{-6}$	$1.26 \cdot 10^{-12}$	$3.29 \cdot 10^{-9}$	$1.66 \cdot 10^{-9}$	$1.70 \cdot 10^{-9}$
	UV disinfection	$9.22 \cdot 10^{-5}$	$2.04 \cdot 10^{-4}$	$1.32 \cdot 10^{-4}$	$1.42 \cdot 10^{-4}$	$1.84 \cdot 10^{-6}$	$4.05 \cdot 10^{-6}$	$2.62 \cdot 10^{-6}$	$2.82 \cdot 10^{-6}$	$1.04 \cdot 10^{-9}$	$3.49 \cdot 10^{-9}$	$1.97 \cdot 10^{-9}$	$2.16 \cdot 10^{-9}$
Field-obtained	Chemical disinfection	$1.11 \cdot 10^{-5}$	$3.52 \cdot 10^{-3}$	$5.50 \cdot 10^{-4}$	$1.52 \cdot 10^{-3}$	$2.21 \cdot 10^{-7}$	$7.01 \cdot 10^{-5}$	$1.09 \cdot 10^{-5}$	$3.02 \cdot 10^{-5}$	$1.47 \cdot 10^{-10}$	$5.79 \cdot 10^{-8}$	$7.45 \cdot 10^{-9}$	$2.31 \cdot 10^{-8}$
	Filtration	$2.72 \cdot 10^{-7}$	$2.62 \cdot 10^{-4}$	$1.20 \cdot 10^{-5}$	$7.19 \cdot 10^{-4}$	$5.41 \cdot 10^{-9}$	$5.22 \cdot 10^{-6}$	$2.39 \cdot 10^{-7}$	$1.43 \cdot 10^{-5}$	$3.98 \cdot 10^{-12}$	$3.87 \cdot 10^{-9}$	$1.74 \cdot 10^{-10}$	$1.09 \cdot 10^{-8}$
	UV disinfection	$1.25 \cdot 10^{-6}$	$2.80 \cdot 10^{-3}$	$7.48 \cdot 10^{-4}$	$1.17 \cdot 10^{-3}$	$2.49 \cdot 10^{-8}$	$5.58 \cdot 10^{-5}$	$1.49 \cdot 10^{-5}$	$2.33 \cdot 10^{-5}$	$1.87 \cdot 10^{-11}$	$4.42 \cdot 10^{-8}$	$1.14 \cdot 10^{-8}$	$1.78 \cdot 10^{-8}$
Difference between manufacturer-claimed and field-obtained LRV scenarios	Chemical disinfection	$-1.29 \cdot 10^{-4}$	$3.43 \cdot 10^{-3}$	$4.38 \cdot 10^{-4}$	$1.42 \cdot 10^{-3}$	$-2.57 \cdot 10^{-6}$	$6.82 \cdot 10^{-5}$	$8.71 \cdot 10^{-6}$	$2.82 \cdot 10^{-5}$	$-2.03 \cdot 10^{-9}$	$5.66 \cdot 10^{-8}$	$5.71 \cdot 10^{-9}$	$2.16 \cdot 10^{-8}$
	Filtration	$-1.67 \cdot 10^{-4}$	$1.32 \cdot 10^{-4}$	$-8.55 \cdot 10^{-5}$	$6.07 \cdot 10^{-4}$	$-3.32 \cdot 10^{-6}$	$2.62 \cdot 10^{-6}$	$-1.70 \cdot 10^{-6}$	$1.21 \cdot 10^{-5}$	$-2.78 \cdot 10^{-9}$	$1.90 \cdot 10^{-9}$	$-1.03 \cdot 10^{-9}$	$9.25 \cdot 10^{-9}$
	UV disinfection	$-1.30 \cdot 10^{-4}$	$2.65 \cdot 10^{-3}$	$5.96 \cdot 10^{-4}$	$1.03 \cdot 10^{-3}$	$-2.59 \cdot 10^{-6}$	$5.27 \cdot 10^{-5}$	$1.19 \cdot 10^{-5}$	$2.05 \cdot 10^{-5}$	$-1.94 \cdot 10^{-9}$	$4.17 \cdot 10^{-8}$	$9.00 \cdot 10^{-9}$	$1.56 \cdot 10^{-8}$

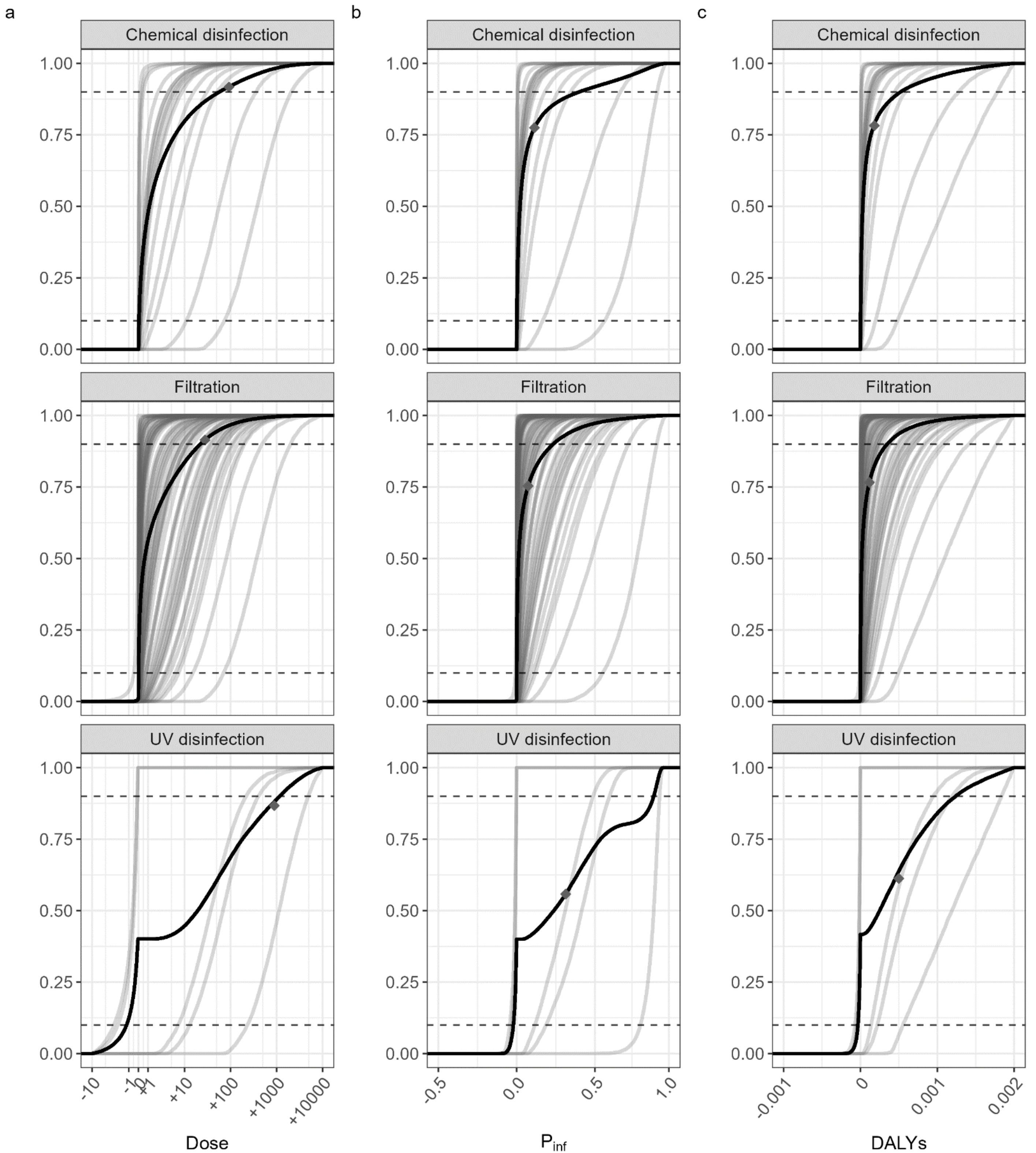

Figure S6.6: Subtraction of C. jejuni *endpoints to compare QMRA scenarios based on manufacturer-claimed and field-obtained LRVs. Positive values indicate that field-based endpoints are higher than their laboratory-based counterparts. Endpoints examined are a) dose of* C. jeujeni *ingested via drinking water per 7-day backpacking trip; b) probability of infecction with* C. jeujeni *via drinking water per 7-day backpacking trip; and c) DALYs arising from ingestion of* C. jeujeni *via drinking water per 7-day backpacking trip. Darker lines represent compiled results for each treatment category; diamond shapes represent the mean endpoint values for compiled results. Dashed lines represent 10% and 90% percentiles for visual aid.*

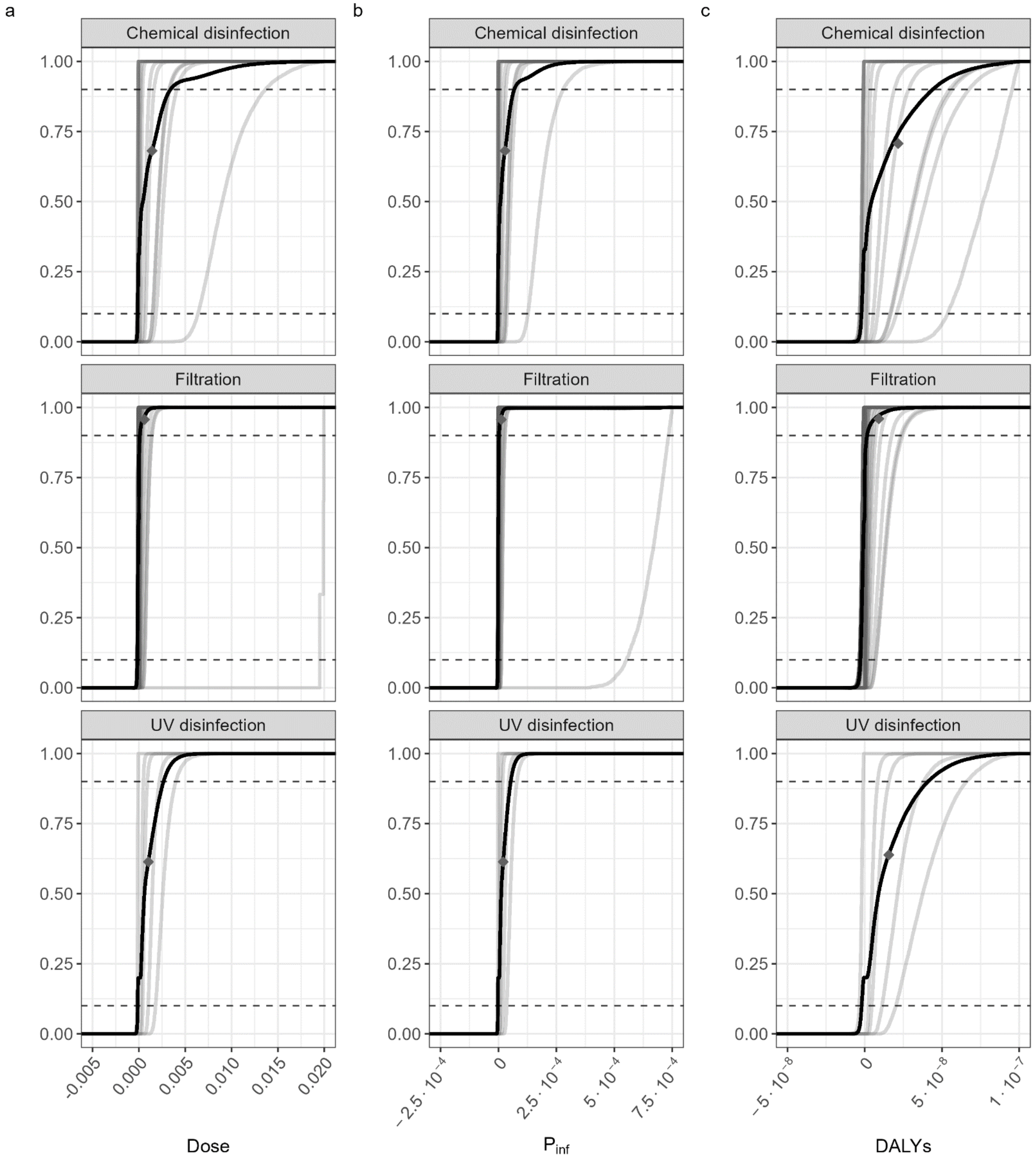

Figure S6.7: Subtraction of G. lamblia *endpoints to compare QMRA scenarios based on manufacturer-claimed and field-obtained LRVs. Positive values indicate that field-based endpoints are higher than their laboratory-based counterparts. Endpoints examined are a) dose of* G. lamblia *ingested via drinking water per 7-day backpacking trip; b) probability of infecction with* G. lamblia *via drinking water per 7-day backpacking trip; and c) DALYs arising from ingestion of* G. lamblia *via drinking water per 7-day backpacking trip. Darker lines represent compiled results for each treatment category; diamond shapes represent the mean endpoint values for compiled results. Dashed lines represent 10% and 90% percentiles for visual aid.*

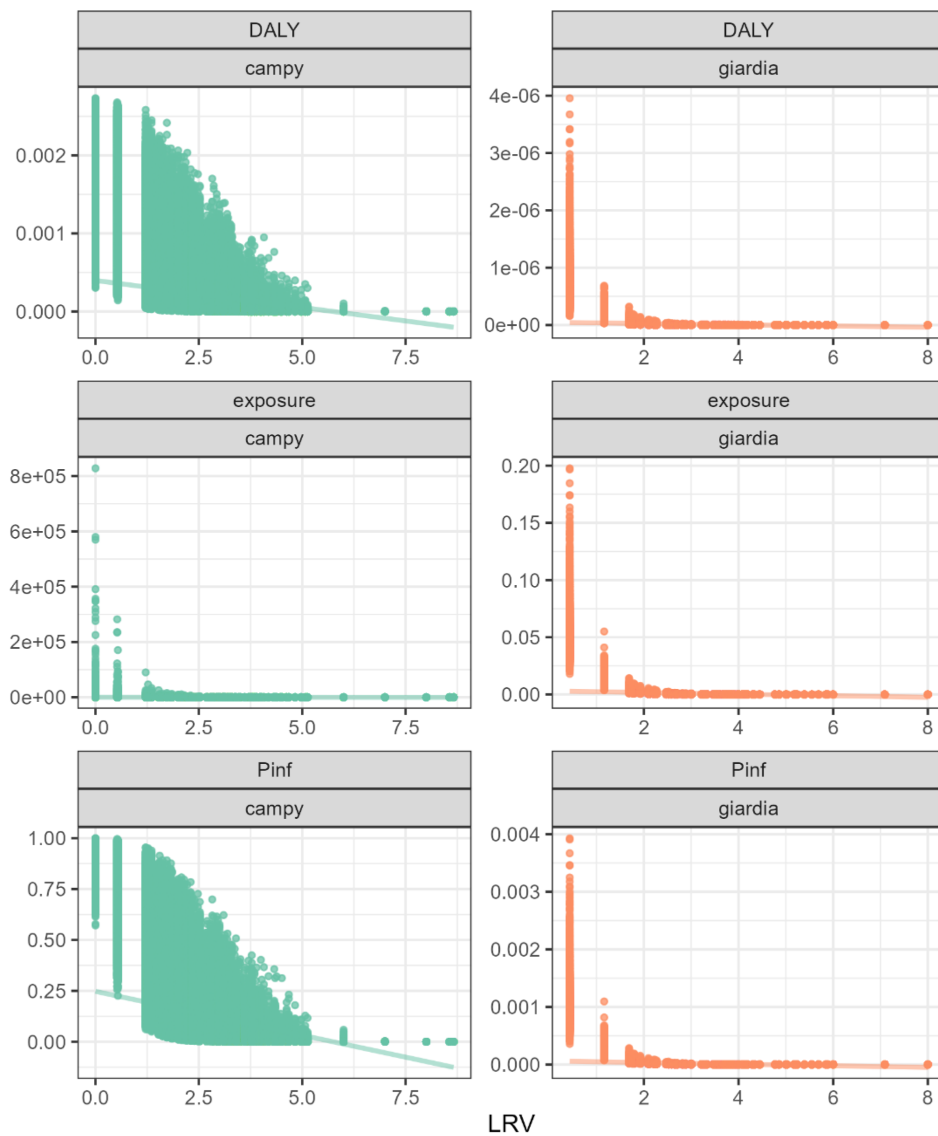

Figure S6.8: Results of sensitivity analysis to correlate QMRA endpoints with input LRVs.

Chapter 7: Overall discussion

7.1 Chapter synthesis

To establish the project space of this dissertation, we examined and summarized the evidence of a discrepancy in performance of POUWT methods under evaluation in the laboratory setting compared to real-use conditions (Chapter 2). This was to support the LRV comparison estimates published by the WHO[19,42], which were not systematically derived. In response to evidence that POUWT devices perform more poorly under real use conditions in comparison to laboratory evaluations, we proposed a method by which POUWT technologies could be evaluated *in situ*: the field challenge test. Under this proposed method, water would be spiked with food-safe probiotic bacteria (EcN, *E. coli* Nissle) and baker's yeast (*S. cerevisiae*) as surrogates for pathogenic bacteria and protozoa, respectively.

In Chapter 3 through Chapter 5, we provided evidence to support the novel use of food-safe spike microbes in application to field challenge testing. In Chapter 3 and Chapter 4, we undertook laboratory work to validate the use of EcN against the commonly-used laboratory strain *E. coli* K-12 (EcK12), in application to disinfection and filtration treatment methods, respectively. The two *E. coli* strains performed relatively similarly, and although there were some statistically significant differences in cell morphology, they were not great enough in magnitude to preclude the use of EcN as a practical surrogate for EcK12 for field challenge testing. In Chapter 5, we reviewed and synthesized published literature investigating the disinfection properties of *S. cerevisiae* in water, which had not yet been reviewed and assessed in comparison to protozoan pathogens. Our meta-analysis indicated that *S. cerevisiae* is a conservative surrogate for *G. lamblia* and *C. parvum* under exposure to UV radiation. There was not enough published data to reach conclusions regarding chemical disinfection (a limitation to our work, discussed below).

In Chapter 6, we used the validated EcN and *S. cerevisiae* surrogates to implement the innovative field challenge test technique. In the summer of 2021, 144 one-on-one surveys were conducted of backcountry campers on Sombrio Beach, in the Juan de Fuca Provincial Park. Each survey consisted of a challenge test of the participants' own water treatment

device, a questionnaire, a 100 mL grab sample of the participants' drinking water, and a hand rinse sample measuring microbiological hand contamination. Field-based LRVs were calculated and compared to manufacturer-claimed LRVs; bacterial reductions were significantly lower in the field than claimed by manufacturers, but *S. cerevisiae* reductions were sometimes similar or occasionally higher than claimed. Field-obtained and manufacturer-claimed LRVs were used as inputs into a QMRA model to simulate and compare pathogen dose values and corresponding health risks. Modelled health risks arising from bacterial pathogens were significantly higher than those arising from protozoan pathogens. When field-based bacterial LRVs were used, health risks were significantly higher than corresponding manufacturer claims. The novel field challenge technique and resulting QMRA analysis provides a framework within which other future field-based LRV data can be contextualized.

7.2 Limitations

There are assorted limitations of this study, some of which could offer opportunities for further research.

7.2.1 Lack of a viral surrogate

The viral pathogen class can be prevalent in surface, ground and drinking water[409], thereby potentially causing infection in vulnerable individuals even if ingested at low concentrations[410,411]. Viruses are the smallest waterborne pathogen class, typically between 10 and 100 nm in size, making them difficult to treat via filtration methods[51]. Although it is critically important to address viruses when taking measures to prevent waterborne illness[412], it was not feasible to address food-safe viral surrogates within the scope of my PhD work. There is not yet a food-safe viral surrogate available that would be appropriate for use in field challenge testing, and this is a gap that could be filled with further research.

Non-infectious "virus-like particles" (VLPs; also referred to as "mock virus" particles) are a synthesized assembly of proteins (capsomeres) further organised into a nanoparticle (capsid)[413], which have been investigated as surrogates to evaluate performance of membrane-based water treatment processes at the bench scale[413,414]. VLPs designed to be morphologically and antigenically similar to norovirus were successfully used to test laboratory-scale conventional coagulation-rapid sand filtration in application to water treatment[415,416].

It is not yet clear whether VLPs could be considered appropriate for field challenge testing, but they, or a similar particle, could be potential candidates. VLPs are inherently unable to replicate[413,414,417], and are being explored as a novel approach to vaccine development[417–421], potentially making them safe for human exposure as would be required for field challenge testing[20]. Although a promising idea, protocols to produce and enumerate VLPs are currently firmly laboratory-based (i.e., enumeration via enzyme-linked immunosorbent assay, ELISA, or real-time polymerase chain reaction, RT-PCR)[422]. Field-appropriate methods would need development before implementation at any scale, although viral enumeration techniques that can be performed in the field or in low-resource contexts are the focus of recent progress (i.e., LAMP, loop-mediated isothermal amplification)[422–424] and could plausibly be applied to enumerate VLPs.

7.2.2 POUWT technology selection for microbe validation

As outlined in Section 1.3, there are many approaches available for POUWT, with filtration (granular or membrane), chlorination, UV disinfection, combined filtration/chlorination, and combined flocculation/chlorination as the most common options[52]. Of these, we examined filtration (specifically ceramic filtration, Chapter 4), chlorination (specifically free chlorine disinfection, Chapter 3 and Chapter 5) and UV disinfection (specifically in the UV-C wavelength range, Chapter 3 and Chapter 5).

Given the wide range of POUWT technologies and the limitations in PhD scope, we did not examine the field-appropriate surrogates EcN and *S. cerevisiae* in response to granular media filtration (i.e., rapid sand filtration or BSF), membrane filtration including nano- or microfiltration, or combination treatments (i.e., filtration/chlorination or flocculation/chlorination). However, we did investigate the morphological characteristics of EcN (i.e., particle size and zeta potential, Chapter 4) in comparison to standard laboratory strain *E. coli* K-12, which were statistically different but functionally similar (i.e., relatively small mean differences observed in particle size and zeta potential). Therefore, we could reasonably expect that EcN would be an appropriate bacterial surrogate for use in challenge testing other filtration-based methods than CWF.

S. cerevisiae has been validated for filtration in comparison to protozoan pathogens elsewhere[37], and is similar in size to protozoan pathogens – approximately 5 – 10 µm in length[425] in comparison to *G. lamblia* (approximately 5 – 15 µm) and *C. parvum* (approximately 3 – 7 µm)[51]. Zeta potential is also reasonably similar between *S. cerevisiae*

and protozoan pathogens of concern[37] – approximately -30 mV in distilled water at pH 6.5[426], in comparison to *G. lamblia* (approximately -13 to -20 mV) and *C. parvum* (approximately -37 to -42 mV)[427]. Zeta potential is highly dependant on pH and water conductivity conditions and tends to decrease with increasing ionic strength (conductivity) of the water[427]. This was observed for *S. cerevisiae*, with zeta potential values dropping to as low as -6.4 mV with the introduction of calcium chloride ($CaCl_2$) ions[426].

Due to the morphological similarity of EcN and *S. cerevisiae* with their respective analogs, it would be reasonable to expect that they would also perform similarly in response to other filtration methods that operate on physical treatment principles. With respect to rapid sand filtration, which utilizes depth filtration, the similarity in size and zeta potential would likely mean that EcN and *S. cerevisiae* would be retained at similar levels as EcK12 and protozoan pathogens, respectively. Membrane filtration and BSF methods that mainly rely on surface straining and cake filtration (i.e., reliant on particle size as the retention mechanism) would also respond analogously due to the particle size similarities.

Treatment of water at the point of use via coagulation and flocculation is not commonly used in the hiking/backcountry setting but is employed in low-resource contexts[52]. Coagulation takes place by the addition of hydrolyzing chemicals such as alum or iron salts and/or polymers to destabilize suspended particulate matter, including pathogens, so that they can aggregate and form flocs, thus making them easier to settle or filter[51]. The relative stability of particles in water is influenced by the surface charge (i.e., zeta potential), and therefore we would expect EcN and *S. cerevisiae* to respond similarly to coagulation/flocculation as their respective analogs.

7.2.3 Water matrix effects

In laboratory testing of POUWT devices, the WHO[19] recommends using a "worst-case" challenge water, with high turbidity, pH and low temperature, in addition to a "best-case" water of relatively high quality. This is to rigorously demonstrate that a POUWT device will maintain its ability to achieve specified microbe elimination targets under challenging field or consumer use conditions[112].

However, given limitations in time and scope of my PhD work, we did not investigate the effects of water matrix conditions on the response of the food-safe surrogates we used to water treatment mechanisms. In laboratory work to compare EcN to EcK12 in response

to disinfection (Chapter 3) and filtration (Chapter 43.6), we only used high-quality mineral water or Butterfield's phosphate buffer. Low-quality test water is recommended to be used for additional tests per WHO[19], with a high (> 30 NTU) turbidity, low (4°C) temperature and variable (6-10) pH, to investigate POUWT efficacy under challenging matrix conditions. Given morphological similarities and comparability in the results of our experimental work, it would be reasonable to expect that EcN and EcK12 would also respond similarly to water treatment if placed in low-quality water, however this would need to be confirmed with further laboratory investigation. In our literature review to validate *S. cerevisiae* as a protozoan surrogate (Chapter 5), we were confined to analysing the effects of water conditions as published in the literature, which were typically conducted in high-quality distilled, mineral or phosphate buffered water. Therefore, the response of *S. cerevisiae* to worst-case water, and any resulting comparisons to protozoan pathogens, would need to be explored further.

7.2.4 Behaviour of *S. cerevisiae* in response to free chlorine for PoU treatment

There was a paucity of studies identified via our literature review (Chapter 5) that studied chemical disinfection methods for *S. cerevisiae* inactivation. In particular, free chlorine disinfection is a cost-effective[133] and proven method to improve water quality in development contexts[61]. Given the applicability of free chlorine disinfection to POUWT and low-resource contexts, it would be pertinent to investigate the dose-response relationship of *S. cerevisiae* to free chlorine disinfection. This would complete the "suite" of information regarding the behaviour of *S. cerevisiae* in response to the major categories of POUWT: filtration[37,38], UV disinfection (Chapter 5) and free chlorine disinfection.

7.2.5 Inherent shortcomings of challenge testing

Culture-based enumeration methods

The culture-based enumeration methods we used for *E. coli* and *S. cerevisiae* were appropriate for field and low-resource settings due to the pre-packaged sterile materials they use, as well as the lack of requirement for electricity for reagent refrigeration[188] and incubation[359]. Despite their wide use, these and other culture-based methods do not quantify viable but non-culturable (VBNC) cells.

The phenomenon of VBNC cells, particularly in bacteria, is characterized by a loss of culturability using conventional plate count techniques, typically due to exposure to various stresses[428]. Although cells may be injured or otherwise stressed and thus do not exhibit growth in response to typical culture-based enumeration methods, they may still be infective, or resuscitated back to an infective or culturable state[428]. In response to disinfection, including UV and free chlorine disinfection as applied to POUWT, VBNC cells can be produced, which could result in overestimation of the effectiveness of treatment[112]. Methods to quantify VBNC bacteria include use of a differential staining procedure and subsequent direct microscopic enumeration; flow cytometry, which also utilizes different fluorescent dyes but enumerates dyed cells by laser-optical methods; or detection of gene expression by reverse transcription polymerase chain reaction (RT-PCR), detecting mRNA which have a short half-life and thus indicate the presence of viable cells[428,429]. These methods require laboratory-based equipment and their applicability to field-based challenge testing is therefore limited.

Although VBNC cells do present a shortcoming towards the field-based culture methods we used in the present work, laboratory-based challenge testing guidelines also recommend use of culture-based enumeration methods[18,19], and most laboratory-based protocols do not mention VBNC issues at all[112]. Therefore, the same limitation applies to both field- and laboratory-based challenge testing, making comparisons within and between the two reasonable. Field-based methods to quantify VBNC bacteria remain an open topic of interest, particularly in relation to on-site and in-line water quality monitoring[429].

Limitations of LRVs

Achievement of high LRVs in the laboratory setting does not translate into high LRVs when POUWT technologies are used in practice. This “laboratory versus field” gap is discussed in Chapter 2 and the field challenge test concept developed in my PhD work was aimed at quantifying this gap. However, even if high LRVs are achieved by POUWT methods when measured *in situ*, this may not necessarily translate into tangible health gains, for several reasons.

Once treated, drinking water must also be safely stored and drunk from a clean vessel to protect against recontamination, which can undo the benefits of water treatment[78,79,85,86,430]. The highest health gains occur when water is collected from an improved source of adequate quality[77] using a clean fetching container[78,79]; treated

consistently[31–35] and correctly[21,25,81–84]; and stored using a clean vessel after treatment, then drunk using a clean cup[78,79,85,86,430]. To sustainably achieve safe water access, we must take the "source to sip" approach, which includes all these important components[15].

Even one day per year spent drinking untreated water can dramatically increase the risk of infection and subsequent waterborne illness[431], and therefore adoption is critically important in realizing the protective benefits of POUWT[31–35]. Technologies that achieve high LRVs, even under field conditions, are not useful if they are not used. Adoption of POUWT methods requires users to invest time and often money into a process whose benefit occurs in the *absence* of diarrhea. Time and money can be in short supply even for households in high-income countries; in low-resource areas it may not be feasible to expect users to spend it treating their water, which is something not generally required in high-resource areas. The poorest typically pay disproportionally more for water[432,433] and the added time and energy burden required to treat water acts as a "bandwidth tax"[12] that can be overwhelming when added to existing stresses of managing and rationing water in water-deprived areas alongside day-to-day household chores and anxieties[434]. The unpaid domestic chores of fetching, treating and storing water primarily falls to women and girls[433,435,436], who disproportionately bear this burden on their time, energy and money[12].

There have been calls to evaluate HWT approaches in terms of their affordability, labour burden (especially regarding women and girls), user acceptance/adoption, health impacts and potential technical performance (i.e., LRVs achieved in the laboratory or field), rather than purely from a technical and/or cost perspective, as has often been the case with past interventions[12]. Indeed, implementation of POUWT interventions can succeed or fail based on barriers and enablers in five main domains per Chaúque et al.[437], most of which do not involve $\log_{10}$ microbe reductions: psychosocial factors (i.e., acceptance of technology or attitudes towards water treatment), promotional approaches (i.e., stakeholder motivation, technology introduction and training strategies), technology selection (i.e., contextual appropriateness and long-term operability), economic viability (i.e., acquisition and maintenance costs), and environmental factors (i.e., water quality impacts).

7.3 Directions for future work

7.3.1 Extending field challenge test applications

There are more opportunities for further application of the field challenge test method. In Chapter 6 we combined challenge testing with a participant questionnaire, hand rinse to measure microbiological contamination and water quality testing of participants' personal water. We took a focussed approach because our objective was to develop the challenge test method, however the technique could be incorporated into a larger, comprehensive household survey following the format of the Multiple Indicator Cluster Surveys (MICS)[113,115]. MICS additionally collects survey information on water supply, sanitation and hygiene, sending specifically-trained enumerators (either researchers, or local employees) to visit local households. Using MICS survey techniques combined with the field challenge test method outlined in Chapter 6, context-specific estimates can be gathered of the microbiological performance of POUWT methods. Context-specific LRV data could also be viewed as a part of a larger picture that is painted by overall survey responses.

Although we were not able to undertake such an analysis due to the relatively low sample size for each brand of device that was studied during our fieldwork (Chapter 6), with a more focussed study, explanatory factors for compromised POUWT performance could be investigated. Many such factors have already been identified, for example cracking or damage of ceramic filter elements allowing short-circuiting[24,25,100,101]; incorrect or inconsistent chlorine dosage[81]; or variable or poor source water quality hindering treatment[24,28,81]. In most cases the specific impact(s) of such factor(s) on resulting treatment LRVs have not been quantified. Using field challenge testing in conjunction with a structured user observation and comprehensive interview, it might be possible to quantify the impact of context-specific factors (e.g., user behaviour, water quality) on treatment LRV. The ability to associate explanatory factors with LRV impacts has several promising implications.

First, factors with the highest effect (either positive or negative) on treatment LRV could be identified, and the corresponding drop or gain in health risk calculated via a QMRA analysis. For example, it could be possible to produce the following statement: "*Use of low batteries for UV-LED disinfection was correlated with an average 2log_{10} (two orders of*

magnitude) drop in treatment performance and thus increased the probability of infection attributable to drinking water consumption by 2.25 times".

Interventions to improve performance of POUWT methods could be prioritized based on field evidence, which could also be used to develop instructional or advertising campaigns for best practices for water treatment, or to inform local support systems of the best allocation of resources. With respect to the UV-LED example, if use of low batteries is identified as the factor having the highest negative impact on performance, then informational campaigns could be initiated, encouraging individuals to check their batteries, together with procuring an adequate and affordable supply of batteries. Field-gathered LRVs could also be used to guide design of POUWT technologies or evaluate manufacturer-provided instructions (e.g., design the UV-LED to flash a warning light if batteries are low).

Potentially, if sufficient data are gathered, a list of the riskiest (or most protective) factors affecting POUWT performance could be generated. Such a list may be context-specific but would still be instructive to prioritize appropriate interventions. An excellent example of a similar practice can be found within the food processing supply chain, which uses the well-established hazard analysis critical control point (HACCP) framework[438,439]. The HACCP system seeks to identify hazards associated with any stage of food production and assess the related risks, with the aim to determine junctions (i.e., critical control points) where targeted risk control procedures would be most effective[439]. Guidelines on developing critical control points are well integrated and there are many resources available on this topic (e.g., by the Canadian Food Inspection Agency[440]).

Recently, there have been developments towards testing and implementing a structured sanitary inspection/survey in application to POUWT methods[400,401]. Such an inspection would be based on established sanitary inspection methods for evaluating drinking water sources[402], which provides a point-by-point guide to assess the health risk factors of a given water source. The POUWT sanitary survey would be intended to augment existing techniques to evaluate household water treatment and safe storage approaches, for example use of the core HWTS indicators[120] and the more general household practices sanitary inspection form[116]. Potential quantification of the riskiest (or most protective) factors in field performance of POUWT devices, as proposed here, would help to validate specific items on the proposed POUWT sanitary inspection checklist. This would provide an

evidence-based way to support the design and inclusion/exclusion of specific survey criteria into the sanitary inspection.

7.3.2 On-site quality control of ceramic water filters

Aside from interview-based applications to enumerate $\log_{10}$ reductions of POUWT as operated by end users, field challenge testing can also be used in application to ceramic water filter (CWF) manufacturing and quality control, partially discussed in Chapter 4. CWFs are typically locally produced at decentralised facilities, which has advantages including simplicity, small business opportunities and local supply chain access[57]. A drawback of such open-source production is variable quality control which has been documented both across and within manufacturing facilities[124,203], potentially resulting in CWFs of variable quality and performance efficacies[124]. Scaling up the production and use of CWFs to promote more widespread delivery of safe drinking water is inhibited by a lack of universal quality control procedures[441], which in turn is inhibited by lack of access to a laboratory, equipment and/or trained personnel[124].

Field-based techniques that do not require an on-site laboratory or electricity supply would be well-suited to the decentralized environment in which CWFs are manufactured. Using the food-safe surrogates EcN and *S. cerevisiae*, manufacturers could conduct on-site challenge tests of CWFs as a method of quality control or to facilitate new designs. On-site microbiological quality control could be incorporated into broader CWF testing standards, allowing accurate comparisons of filters between manufacturers or to a standard[441], for example that of the WHO[19]. In-house or local challenge testing would keep quality control and general water treatment activities local to the community, a practice that has been highlighted[442] to be important in sustainable access to safe water.

7.4 Conclusion

The overall aim of this work was to develop a method under which POUWT technologies can be evaluated under real-use conditions. We synthesized evidence that POUWT devices perform more poorly under real use conditions in comparison to laboratory evaluations (Specific Objective 1, SO1); we proposed “field challenge testing”, using food-safe probiotic bacteria (EcN, *E. coli* Nissle) and baker’s yeast (*S. cerevisiae*) as surrogates for pathogenic bacteria and protozoa, respectively.

To this end (SO2), we successfully validated the use of probiotic *E. coli* as a surrogate for commonly-used laboratory strain *E. coli* K-12, which itself can be used as a surrogate to represent bacterial pathogens. *S. cerevisiae* was investigated via literature review to serve as a field-appropriate protozoan surrogate. It was found to be a conservative surrogate in application to UV disinfection, however there was not enough published data to validate *S. cerevisiae* under chemical disinfection. The food-safe surrogates, probiotic *E. coli* and *S. cerevisiae*, were deployed to conduct field-based challenge testing of POUWT devices in a novel study conducted along the Juan de Fuca backpacking trail in British Columbia, Canada (SO3).

Using field challenge testing, we were able to ascertain the performance of POUWT methods under real-use conditions. Our field-based LRVs were generally lower than claimed by POUWT device manufacturers for the bacterial microbe class, but for the protozoan microbe class, LRVs were similar to those claimed by manufacturers. Using the QMRA framework, health risks were calculated based on field-based LRVs in comparison to manufacturer-claimed LRVs (SO4). When field-based bacterial LRVs were used, health risks were significantly higher than corresponding manufacturer claims. Health risks arising from bacterial pathogen exposure were estimated to be substantially higher than those from protozoans. The framework used here can be applied to contextualize further field challenge testing.

Our novel field challenge test method has numerous implications including validation of POUWT sanitary inspection criteria, quantifying health impacts of contextual factors, to inform technology selection or to support ceramic water filter manufacturers to conduct on-site microbiological control. However, the current project was conducted in the context of backcountry camping conditions due to COVID-19-related travel restrictions, and context-specific data would need to be gathered prior to additional applications.

Bibliography

1. United Nations General Assembly. The human rights to safe drinking water and sanitation: resolution, adopted by the General Assembly. (2016).
2. United Nations. THE 17 GOALS | Sustainable Development. https://sdgs.un.org/goals (2015).
3. Eshetu, E. B. & Woldesenbet, S. A. Are there particular social determinants of health for the world's poorest countries? *African Health Sciences* 11, (2011).
4. Mintz, E., Bartram, J., Lochery, P. & Wegelin, M. Not Just a Drop in the Bucket: Expanding Access to Point-of-Use Water Treatment Systems. *Am J Public Health* 91, 1565–1570 (2001).
5. Prüss-Üstün, A., Bos, R., Gore, F. & Bartram, J. *Safer Water, Better Health.* http://www.who.int/quantifying_ehimpacts/publications/saferwater/en/ (2008).
6. Guerrant, R. L. *et al.* Magnitude and Impact of Diarrheal Diseases. *Archives of Medical Research* 33, 351–355 (2002).
7. Brown, J., Cairncross, S. & Ensink, J. H. J. Water, sanitation, hygiene and enteric infections in children. *Arch Dis Child* 98, 629–634 (2013).
8. Victora, C. G. *et al.* Maternal and child undernutrition: consequences for adult health and human capital. *The Lancet* 371, 340–357 (2008).
9. Graham, J. P., Hirai, M. & Kim, S.-S. An Analysis of Water Collection Labor among Women and Children in 24 Sub-Saharan African Countries. *PLOS ONE* 11, e0155981 (2016).
10. Mitra, A. & Rao, N. Gender, water, and nutrition in India: an intersectional perspective. *Water Alternatives* 12, 169–191 (2019).
11. Shah, S. H. *et al.* Variations in household water affordability and water insecurity: An intersectional perspective from 18 low- and middle-income countries. *Environment and Planning F* 26349825231156900 (2023) doi:10.1177/26349825231156900.
12. Cherukumilli, K., Ray, I. & Pickering, A. J. Evaluating the hidden costs of drinking water treatment technologies. *Nat Water* 1–9 (2023) doi:10.1038/s44221-023-00055-y.
13. UNICEF & WHO. Progress on household drinking water, sanitation and hygiene 2000-2017: special focus on inequalities. (2019).
14. WHO. Drinking Water Key Facts. https://www.who.int/news-room/fact-sheets/detail/drinking-water (2022).
15. Amrose, S., Burt, Z. & Ray, I. Safe Drinking Water for Low-Income Regions. *Annu. Rev. Environ. Resour.* 40, 203–231 (2015).

16. Bhandari, D., Bi, P., Sherchand, J. B., Dhimal, M. & Hanson-Easey, S. Assessing the effect of climate factors on childhood diarrhoea burden in Kathmandu, Nepal. *International Journal of Hygiene and Environmental Health* (2019) doi:10.1016/j.ijheh.2019.09.002.
17. Delpla, I., Jung, A.-V., Baures, E., Clement, M. & Thomas, O. Impacts of climate change on surface water quality in relation to drinking water production. *Environment International* 35, 1225–1233 (2009).
18. USEPA. *Guide standard and protocol for testing microbiological water purifiers.* https://nepis.epa.gov/Exe/ZyNET.exe/9102362E.TXT?ZyActionD=ZyDocument&Client=EPA&Index=1986+Thru+1990&Docs=&Query=&Time=&EndTime=&SearchMethod=1&TocRestrict=n&Toc=&TocEntry=&QField=&QFieldYear=&QFieldMonth=&QFieldDay=&IntQFieldOp=0&ExtQFieldOp=0&XmlQuery=&File=D%3A%5Czyfiles%5CIndex%20Data%5C86thru90%5CTxt%5C00000033%5C9102362E.txt&User=ANONYMOUS&Password=anonymous&SortMethod=h%7C-&MaximumDocuments=1&FuzzyDegree=0&ImageQuality=r75g8/r75g8/x150y150g16/i425&Display=hpfr&DefSeekPage=x&SearchBack=ZyActionL&Back=ZyActionS&BackDesc=Results%20page&MaximumPages=1&ZyEntry=1&SeekPage=x&ZyPURL (1987).
19. WHO. *Evaluating Household Water Treatment Options: Health based targets and Microbiological Performance Specifications.* https://scholar.google.ca/scholar?hl=en&as_sdt=0%2C5&q=Evaluating+Household+Water+Treatment+Options+Health+based+targets+and+microbiological+performance+specifications&btnG= (2011).
20. Zimmer, C. & Dorea, C. C. Differences in laboratory versus field treatment performance of point-of-use drinking water treatment methods: research gaps and ways forward. *npj Clean Water* 6, 1–7 (2023).
21. Karim, M. R. *et al.* Microbiological effectiveness of mineral pot filters as household water treatment in the coastal areas of Bangladesh. *Microbial Risk Analysis* 4, 7–15 (2016).
22. Levy, K. *et al.* Household effectiveness vs. laboratory efficacy of point-of-use chlorination. *Water Research* 54, 69–77 (2014).
23. Meierhofer, R., Banziger, C., Deppeler, S., Kunwar, B. M. & Bhatta, M. From Water Source to Tap of Ceramic Filters-Factors That Influence Water Quality Between Collection and Consumption in Rural Households in Nepal. *Int. J. Environ. Res. Public Health* 15, 2439 (2018).
24. Murray, A. L. *et al.* Evaluation of consistent use, barriers to use, and microbiological effectiveness of three prototype household water treatment technologies in Haiti, Kenya, and Nicaragua. *Science of The Total Environment* 718, 134685 (2020).

25. Murray, A. L. *et al.* Laboratory efficacy and field effectiveness of hollow fiber membrane microfilters used for household water treatment in Honduras. *Journal of Water Sanitation and Hygiene for Development* 7, 74–84 (2017).
26. Neumann, A. *et al.* Arsenic Removal with Composite Iron Matrix Filters in Bangladesh: A Field and Laboratory Study. *Environ. Sci. Technol.* 47, 4544–4554 (2013).
27. Sangsanont, J., Dan, D. T., Nga, T. T. V., Katayama, H. & Furumai, H. Detection of pepper mild mottle virus as an indicator for drinking water quality in Hanoi, Vietnam, in large volume of water after household treatment. *Journal of Environmental Science and Health, Part A* 51, 1100–1106 (2016).
28. Stauber, C. E. *et al.* Characterisation of the biosand filter for E. coli reductions from household drinking water under controlled laboratory and field use conditions. *Water Science and Technology* 54, 1–7 (2006).
29. White, C. *et al. Effect of Contaminated Filtration Sand on Performance of Household Biosand Filters.* (Ieee, 2013).
30. Enger, K. S., Nelson, K. L., Clasen, T., Rose, J. B. & Eisenberg, J. N. S. Linking Quantitative Microbial Risk Assessment and Epidemiological Data: Informing Safe Drinking Water Trials in Developing Countries. *Environ. Sci. Technol.* 46, 5160–5167 (2012).
31. Bivins, A. *et al.* Selecting Household Water Treatment Options on the Basis of World Health Organization Performance Testing Protocols. *Environ. Sci. Technol.* 53, 5043–5051 (2019).
32. Brown, J. & Clasen, T. High Adherence Is Necessary to Realize Health Gains from Water Quality Interventions. *PLOS ONE* 7, e36735 (2012).
33. Brown, J., Hayashi, M. A. L. & Eisenberg, J. N. S. The Critical Role of Compliance in Delivering Health Gains from Environmental Health Interventions. *The American Journal of Tropical Medicine and Hygiene* 100, 777–779 (2019).
34. Enger, K. S., Nelson, K. L., Rose, J. B. & Eisenberg, J. N. S. The joint effects of efficacy and compliance: A study of household water treatment effectiveness against childhood diarrhea. *Water Research* 47, 1181–1190 (2013).
35. Hayashi, M. A. L., Eisenberg, M. C. & Eisenberg, J. N. S. Linking Decision Theory and Quantitative Microbial Risk Assessment: Tradeoffs Between Compliance and Efficacy for Waterborne Disease Interventions. *Risk Analysis* 0, (2019).
36. Zimmer, C. Innovative techniques for the quantification of waterborne microbial risks in field studies. (2019).
37. Chung, J. Development of fluorescently labelled Cryptosporidium oocyst surrogates to test the efficacy of sand filtration processes. (University of New South Wales, 2012).

38. Liu, L. Direct filtration of Cryptosporidium surrogates in drinking water treatment-a multiscale approach. *ERA* https://era.library.ualberta.ca/items/c086ceee-fa2d-4a9f-b9f6-6fddae139ac6 (2019) doi:10.7939/r3-w5dz-0997.
39. Davies, C. M. *et al.* Microbial challenge-testing of treatment processes for quantifying stormwater recycling risks and management. *Water Science and Technology* 57, 843–847 (2008).
40. Shoults, D. C. *et al.* Pathogen performance testing of a natural swimming pool using a cocktail of microbiological surrogates and QMRA-derived management goals. *Journal of Water and Health* jwh2021015 (2021) doi:10.2166/wh.2021.015.
41. Bichai, F. & Ashbolt, N. Public health and water quality management in low-exposure stormwater schemes: A critical review of regulatory frameworks and path forward. *Sustainable Cities and Society* 28, 453–465 (2017).
42. WHO. *Managing Water in the Home: Accelerated Health Gains from Improved Water Supply.* http://www.who.int/water_sanitation_health/dwq/WSH02.07.pdf (2002).
43. Rosa, G. & Clasen, T. Estimating the Scope of Household Water Treatment in Low- and Medium-Income Countries. *Am J Trop Med Hyg* 82, 289–300 (2010).
44. Welch, T. E. Risk of giardiasis from consumption of wilderness water in North America: A systematic review of epidemiologic data. *International Journal of Infectious Diseases* 4, 100–103 (2000).
45. Tallon, P., Magajna, B., Lofranco, C. & Leung, K. T. Microbial Indicators of Faecal Contamination in Water: A Current Perspective. *Water Air Soil Pollut* 166, 139–166 (2005).
46. Boulware, D. R., Forgey, W. W. & Martin, W. J. Medical risks of wilderness hiking. *The American Journal of Medicine* 114, 288–293 (2003).
47. Crouse, B. J. Health Care Needs of Appalachian Trail Hikers. *The Journal o f Family Practice* 36, 5 (1993).
48. Zell, S. C. Epidemiology of wilderness-acquired diarrhea: implications for prevention and treatment. *Journal of Wilderness Medicine* 3, 241–249 (1992).
49. BC Assembly of First Nations. Pacheedaht First Nation. https://www.bcafn.ca/first-nations-bc/vancouver-island-coast/pacheedaht-first-nation (2023).
50. BC Parks. Juan de Fuca Provincial Park Map and Information Guide. (2022).
51. Crittenden, J. C. *et al. MWH's Water Treatment Principles and Design.* (John Wiley & Sons, 2012).
52. Lantagne, D. S., Quick, R. & Mintz, E. D. Household Water Treatment and Safe Storage Options in Developing Countries: A Review of Current Implementation Practices. *Navig* 17 (2006).
53. Freitas, B. L. S. *et al.* A critical overview of household slow sand filters for water treatment. *Water Research* 208, 117870 (2022).

54. Yang, H., Xu, S., Chitwood, D. E. & Wang, Y. Ceramic water filter for point-of-use water treatment in developing countries: Principles, challenges and opportunities. *Front. Environ. Sci. Eng.* 14, 79 (2020).
55. Netherlands Water Partnership. Smart Disinfection Solutions. (2010).
56. Pooi, C. K. & Ng, H. Y. Review of low-cost point-of-use water treatment systems for developing communities. *npj Clean Water* 1, 1–8 (2018).
57. CMWG. Best practice recommendations for local manufacturing of ceramic pot filters for household water treatment. 187 p.; ill.61 fig.; 12 tab. (2011).
58. Rayner, J. *et al.* The effects of input materials on ceramic water filter efficacy for household drinking water treatment. *Water Supply* 17, 859–869 (2017).
59. Rayner, J. *et al.* Laboratory Investigation into the Effect of Silver Application on the Bacterial Removal Efficacy of Filter Material for Use on Locally Produced Ceramic Water Filters for Household Drinking Water Treatment. *ACS Sustainable Chem. Eng.* 1, 737–745 (2013).
60. Mintz, E. D., Reiff, F. M. & Tauxe, R. V. Safe Water Treatment and Storage in the Home: A Practical New Strategy to Prevent Waterborne Disease. *JAMA* 273, 948–953 (1995).
61. Arnold, B. F. & Colford, J. M. Jr. Treating water with chlorine at Point-of-Use to improve water quality and reduce child diarrhea in developing countries: a systematic review and meta-analysis. *The American Journal of Tropical Medicine and Hygiene* 76, 354–364 (2007).
62. Crider, Y. S., Tsuchiya, M., Mukundwa, M., Ray, I. & Pickering, A. J. Adoption of Point-of-Use Chlorination for Household Drinking Water Treatment: A Systematic Review. *Environ Health Perspect* 131, 016001 (2023).
63. Lindmark, M. *et al.* Passive In-Line Chlorination for Drinking Water Disinfection: A Critical Review. *Environ. Sci. Technol.* 56, 9164–9181 (2022).
64. Branz, A. *et al.* Chlorination of drinking water in emergencies: a review of knowledge to develop recommendations for implementation and research needed. *Waterlines* 36, 4–39 (2017).
65. Clasen, T., Schmidt, W.-P., Rabie, T., Roberts, I. & Cairncross, S. Interventions to improve water quality for preventing diarrhoea: systematic review and meta-analysis. *BMJ* (2007) doi:10.1136/bmj.39118.489931.BE.
66. Luzi, S., Tobler, M., Suter, F. & Meierhofer, R. SODIS manual: Guidance on solar water disinfection. (2016).
67. García-Gil, Á., García-Muñoz, R. A., McGuigan, K. G. & Marugán, J. Solar Water Disinfection to Produce Safe Drinking Water: A Review of Parameters, Enhancements, and Modelling Approaches to Make SODIS Faster and Safer. *Molecules* 26, 3431 (2021).

68. Chatterley, C. & Linden, K. Demonstration and evaluation of germicidal UV-LEDs for point-of-use water disinfection. *Journal of Water and Health* 8, 479–486 (2010).
69. Chu, C., Ryberg, E. C., Loeb, S. K., Suh, M.-J. & Kim, J.-H. Water Disinfection in Rural Areas Demands Unconventional Solar Technologies. *Acc. Chem. Res.* 52, 1187–1195 (2019).
70. Lui, G. Y., Roser, D., Corkish, R., Ashbolt, N. J. & Stuetz, R. Point-of-use water disinfection using ultraviolet and visible light-emitting diodes. *Science of The Total Environment* 553, 626–635 (2016).
71. United Nations. The Human Right to Water and Sanitation. (2010).
72. Bain, R. *et al.* Global assessment of exposure to faecal contamination through drinking water based on a systematic review. *Tropical Medicine & International Health* 19, 917–927 (2014).
73. Prüss-Ustün, A. *et al.* Burden of disease from inadequate water, sanitation and hygiene in low- and middle-income settings: a retrospective analysis of data from 145 countries. *Tropical Medicine & International Health* 19, 894–905 (2014).
74. Troeger, C. *et al.* Estimates of the global, regional, and national morbidity, mortality, and aetiologies of diarrhoea in 195 countries: a systematic analysis for the Global Burden of Disease Study 2016. *The Lancet Infectious Diseases* 18, 1211–1228 (2018).
75. Ritchie, Roser, Mispy & Oritz-Ospina. Measuring progress towards the Sustainable Development Goals. *Our World in Data* https://sdg-tracker.org/water-and-sanitation (2018).
76. WHO & UNICEF. *WHO/UNICEF Joint Monitoring Report.* https://www.who.int/water_sanitation_health/monitoring/jmp2012/en/ (2012).
77. WHO & UNICEF. *Progress on Drinking Water, Sanitation, Sanitation and Hygiene.* https://apps.who.int/iris/bitstream/handle/10665/258617/9789241512893-eng.pdf;jsessionid=330DBC2E9A8FF2511C029264C82AD90C?sequence=1 (2017).
78. Cassivi, A., Tilley, E., Waygood, E. O. D. & Dorea, C. Household practices in accessing drinking water and post collection contamination: A seasonal cohort study in Malawi. *Water Res.* 189, (2021).
79. Makokove, R., Macherera, M., Kativhu, T. & Gudo, D. F. The effect of household practices on the deterioration of microbial quality of drinking water between source and point of use in Murewa district, Zimbabwe. *Journal of Water and Health* 20, 518–530 (2022).
80. Hayashi, M. A. L., Eisenberg, M. C. & Eisenberg, J. N. S. Linking Decision Theory and Quantitative Microbial Risk Assessment: Tradeoffs Between Compliance and Efficacy for Waterborne Disease Interventions. *Risk Analysis* 39, 2214–2226 (2019).
81. McLaughlin, L. A. *et al.* An Observational Study on the Effectiveness of Point-Of-Use Chlorination. *J Environ Health* 71, 48–53 (2009).

82. Kallman, E. N., Oyanedel-Craver Vinka A., & Smith James A. Ceramic Filters Impregnated with Silver Nanoparticles for Point-of-Use Water Treatment in Rural Guatemala. *Journal of Environmental Engineering* 137, 407–415 (2011).
83. Rayner, J., Murray, A., Joseph, M., Branz, A. & Lantagne, D. Evaluation of household drinking water filter distribution programs in Haiti. *Journal of Water, Sanitation and Hygiene for Development* 6, 42–54 (2016).
84. Farrow, C. *et al.* Ceramic Water Filters: A Point-of-Use Water Treatment Technology to Remove Bacteria from Drinking Water in Longhai City, Fujian Province, China. *JOURNAL OF ENVIRONMENTAL INFORMATICS* 32, 63-68–68 (2018).
85. Mugoya, I. *et al.* Rapid Spread of Vibrio cholerae O1 Throughout Kenya, 2005. *American Journal of Tropical Medicine and Hygene* 78, 527–533 (2008).
86. Rufener, S., Mäusezahl, D., Mosler, H.-J. & Weingartner, R. Quality of Drinking-water at Source and Point-of-consumption—Drinking Cup As a High Potential Recontamination Risk: A Field Study in Bolivia. *J Health Popul Nutr* 28, 34–41 (2010).
87. Brown, J., Sobsey, M. D. & Loomis, D. Local Drinking Water Filters Reduce Diarrheal Disease in Cambodia: A Randomized, Controlled Trial of the Ceramic Water Purifier. *The American Journal of Tropical Medicine and Hygiene* 79, 394–400 (2008).
88. Clasen, T. Household Water Treatment and Safe Storage to Prevent Diarrheal Disease in Developing Countries. *Curr Envir Health Rpt* 2, 69–74 (2015).
89. Capone, D., Bivins, A. & Brown, J. Producing ratio measures of effect with quantitative microbial risk assessment. *Risk Analysis* n/a, (2022).
90. Marois-Fiset, J. T., Shaheed, A., Brown, J. & Dorea, C. C. Laboratory evaluation of a new coagulant/disinfectant point-of-use water treatment product for emergencies. *J. Appl. Microbiol.* 121, 892–902 (2016).
91. Marois-Fiset, J.-T., Carabin, A., Lavoie, A. & Dorea, C. C. Effects of Temperature and pH on Reduction of Bacteria in a Point-of-Use Drinking Water Treatment Product for Emergency Relief. *Applied and Environmental Microbiology* 79, 2107–2109 (2013).
92. Légaré-Julien, F., Lemay, O., Vallée-Godbout, U., Bouchard, C. & Dorea, C. C. Laboratory Efficacy and Disinfection by-Product Formation of a Coagulant/Disinfectant Tablet for Point-of-Use Water Treatment. *Water* 10, 1567 (2018).
93. Peter, M. & Harter, M. Selecting household water filters in emergencies: a manual for field evaluation. (2021).

94. Marios-Fiset, J.-T. & Dorea, C. Sachet-type point-of-use (POU) water treatment product comparison for emergencies. in *DELIVERING WATER, SANITATION AND HYGIENE SERVICES IN AN UNCERTAIN ENVIRONMENT* (2013).

95. Pérez-Vidal, A., Diaz-Gómez, J., Castellanos-Rozo, J. & Usaquen-Perilla, O. L. Long-term evaluation of the performance of four point-of-use water filters. *Water Research* 98, 176–182 (2016).

96. Guerrero-Latorre, L. *et al.* Performance of black ceramic water filters and their implementation in rural Ecuador. *J Water San Hyg Dev* 9, 694–702 (2019).

97. White, C. *et al.* Effect of contaminated filtration sand on performance of household biosand filters. in *2013 IEEE Global Humanitarian Technology Conference (GHTC)* 243–247 (2013). doi:10.1109/GHTC.2013.6713688.

98. Meierhofer, R., Rubli, P., Oremo, J. & Odhiambo, A. Does Activated Silver Reduce Recontamination Risks in the Reservoirs of Ceramic Water Filters? *Water* 11, 1108 (2019).

99. Brown, D., Farrow, C., McBean, E. A., Gharabaghi, B. & Beauchamp, J. Advancing performance evaluation standards for household water treatment technologies. *J Water Health* 17, 266–273 (2019).

100. Boisson, S. *et al.* Field Assessment of a Novel Household-Based Water Filtration Device: A Randomised, Placebo-Controlled Trial in the Democratic Republic of Congo. *PLOS ONE* 5, e12613 (2010).

101. Murray, A. *et al.* Fouling in hollow fiber membrane microfilters used for household water treatment. *Journal of Water, Sanitation and Hygiene for Development* 5, 220–228 (2015).

102. Mellor, J., Abebe, L., Ehdaie, B., Dillingham, R. & Smith, J. Modeling the sustainability of a ceramic water filter intervention. *Water Research* 49, 286–299 (2014).

103. Hill, C. L. *et al.* Field and Laboratory Assessment of a New Electrolytic Point-of-Use Water Treatment Technology. *Water* 14, 1077 (2022).

104. Brown, J. & Sobsey, M. D. Microbiological effectiveness of locally produced ceramic filters for drinking water treatment in Cambodia. *Journal of Water and Health* 8, 1–10 (2010).

105. Mellor, J., Kumpel, E., Ercumen, A. & Zimmerman, J. Systems Approach to Climate, Water, and Diarrhea in Hubli-Dharwad, India. *Environ. Sci. Technol.* 50, 13042–13051 (2016).

106. Smeets, P. W. M. H. Quantitative microbial risk assessment (QMRA) to support decisions for water supply in affluent and developing countries. *Water Practice and Technology* (2019) doi:10.2166/wpt.2019.038.

107. Petterson, S. R. Application of a QMRA Framework to Inform Selection of Drinking Water Interventions in the Developing Context. *Risk Analysis* 36, 203–214 (2016).

108. Rosenthal Joshua *et al.* Systems Science Approaches for Global Environmental Health Research: Enhancing Intervention Design and Implementation for Household Air Pollution (HAP) and Water, Sanitation, and Hygiene (WASH) Programs. *Environ. Health Perspect.* 128, 105001 (2020).
109. Wilhelm, N., Kaufmann, A., Blanton, E. & Lantagne, D. Sodium hypochlorite dosage for household and emergency water treatment: updated recommendations. *J Water Health* 16, 112–125 (2018).
110. Rayner, J. Biosand Filter Evalution: Clear Cambodia. (2020).
111. Sinclair, R. G., Rose, J. B., Hashsham, S. A., Haas, C. N. & Gerba, C. P. Criteria for Selection of Surrogates Used To Study the Fate and Control of Pathogens in the Environment. *Appl. Environ. Microbiol.* 78, 1969–1977 (2012).
112. Bailey, E. S. *et al.* Methods, Protocols, Guidance and Standards for Performance Evaluation for Point-of-Use Water Treatment Technologies: History, Current Status, Future Needs and Directions. *Water* 13, 1094 (2021).
113. UNICEF & MICS. Manual for Water Quality Testing. (2017).
114. UNICEF & WHO. Integrating Water Quality Testing into Household Surveys: Thematic report on drinking water. (2020).
115. UNICEF. Water Quality Testing Questionnaire. (2020).
116. WHO. Household Practices Sanitary Inspection Form. (2020).
117. Millipore. SimPlate Yeast and Mold Color Indicator. (2020).
118. Feldsine, P. T., Lienau, A. H., Leung, S. C. & Mui, L. A. Enumeration of total yeasts and molds in foods by the SimPlate Yeast and Mold-Color Indicator method and conventional culture methods: collaborative study. *Journal of AOAC International* 86, 296–313 (2003).
119. Tortorello, M. L. Indicator Organisms for Safety and Quality—Uses and Methods for Detection: Minireview. *Journal of AOAC INTERNATIONAL* 86, 1208–1217 (2003).
120. WHO. *A toolkit for monitoring and evaluating household water treatment and safe storage programmes.* (World Health Organization, 2012).
121. Sobsey, M. D., Stauber, C. E., Casanova, L. M., Brown, J. M. & Elliott, M. A. Point of Use Household Drinking Water Filtration: A Practical, Effective Solution for Providing Sustained Access to Safe Drinking Water in the Developing World. *Environ. Sci. Technol.* 42, 4261–4267 (2008).
122. Zimmer, C. & Dorea, C. Enumeration of Escherichia coli in Probiotic Products. *Microorganisms* 7, 437 (2019).
123. Smeets, P. W. M. H., Rietveld, L. C., van Dijk, J. C. & Medema, G. J. Practical applications of quantitative microbial risk assessment (QMRA) for water safety plans. *Water Science and Technology* 61, 1561–1568 (2010).

124. Rayner, J., Skinner, B. & Lantagne, D. Current practices in manufacturing locally-made ceramic pot filters for water treatment in developing countries. *Journal of Water, Sanitation and Hygiene for Development* 3, 252–261 (2013).
125. Bain, R., Johnston, R. & Slaymaker, T. Drinking water quality and the SDGs. *npj Clean Water* 3, 1–3 (2020).
126. Shepard, Z. & Oyanedel-Craver, V. A review of the impact of testing conditions on the performance and quality control of locally manufactured, point-of-use ceramic water filters. *Environ. Sci.: Water Res. Technol.* (2022) doi:10.1039/D1EW00587A.
127. Ashbolt, N. J., Grabow, W. O. K. & Snozzi, M. Indicators of microbial water quality. in *13 Indicators of microbial water quality* (2011).
128. Owens, C. E. L. *et al.* Implementation of quantitative microbial risk assessment (QMRA) for public drinking water supplies: Systematic review. *Water Research* 115614 (2020) doi:10.1016/j.watres.2020.115614.
129. Ward, B. J. *et al.* Predictive models using "cheap and easy" field measurements: Can they fill a gap in planning, monitoring, and implementing fecal sludge management solutions? *Water Research* 196, 116997 (2021).
130. Bain, R. E. S., Johnston, R., Khan, S., Hancioglu, A. & Slaymaker, T. *Monitoring drinking water quality in nationally representative household surveys: cross-sectional analysis of 20 Multiple Indicator Cluster Surveys 2014-2019.* http://medrxiv.org/lookup/doi/10.1101/2020.09.21.20174862 (2020) doi:10.1101/2020.09.21.20174862.
131. Keusch, G. T. *et al.* Diarrheal Diseases. in *Disease Control Priorities in Developing Countries* (eds. Jamison, D. T. et al.) (The International Bank for Reconstruction and Development / The World Bank, 2006).
132. Bivins, A. W. *et al.* Estimating Infection Risks and the Global Burden of Diarrheal Disease Attributable to Intermittent Water Supply Using QMRA. *Environ. Sci. Technol.* 51, 7542–7551 (2017).
133. Clasen, T., Haller, L., Walker, D., Bartram, J. & Cairncross, S. Cost-effectiveness of water quality interventions for preventing diarrhoeal disease in developing countries. *Journal of Water and Health* 5, 599–608 (2007).
134. WHO. *Quantitative microbial risk assessment: application for water safety management.* (World Health Organization, 2016).
135. McGuigan, K. G. *et al.* Solar water disinfection (SODIS): A review from bench-top to roof-top. *Journal of Hazardous Materials* 235–236, 29–46 (2012).
136. Bolton, J. R., Mayor-Smith, I. & Linden, K. G. Rethinking the Concepts of Fluence (UV Dose) and Fluence Rate: The Importance of Photon-based Units – A Systemic Review. *Photochemistry and Photobiology* 91, 1252–1262 (2015).

137. Butterfield, C. T., Wattie, E., Megregian, S. & Chambers, C. W. Influence of pH and Temperature on the Survival of Coliforms and Enteric Pathogens When Exposed to Free Chlorine. *Public Health Reports (1896-1970)* 58, 1837–1866 (1943).
138. Hach. Coliforms, Total and E. coli. (2017).
139. Hach. Iodometric Method Using Sodium Thiosulfate. (2015).
140. Hach. Chlorine, Free, USEPA DPD Method. (2014).
141. Hach. Chlorine, Total, USEPA DPD Method. (2022).
142. Blaser, M. J., Smith, P. F., Wang, W. L. & Hoff, J. C. Inactivation of Campylobacter jejuni by chlorine and monochloramine. *Appl. Environ. Microbiol.* 51, 307–311 (1986).
143. Bolton, J. R. & Linden, K. G. Standardization of Methods for Fluence (UV Dose) Determination in Bench-Scale UV Experiments. *Journal of Environmental Engineering* 129, 209–215 (2003).
144. Bolton, J. R., Beck, S. & Linden, K. G. Protocol for the determination of fluence (UV dose) using a low-pressure or low-pressure high-output UV lamp in bench- scale collimated beam ultraviolet experiments. 9 (2015).
145. Rennecker, J. L., Kim, J.-H., Corona-Vasquez, B. & MariÑas, B. J. Role of Disinfectant Concentration and pH in the Inactivation Kinetics of Cryptosporidium parvum Oocysts with Ozone and Monochloramine. *Environ. Sci. Technol.* 35, 2752–2757 (2001).
146. Chick, H. An Investigation of the Laws of Disinfection. *Epidemiology & Infection* 8, 92–158 (1908).
147. Watson, H. E. A Note on the Variation of the Rate of Disinfection with Change in the Concentration of the Disinfectant. *Epidemiol. Infect.* 4, 536–542 (1908).
148. Gard, S. Chemical Inactivation of Viruses. in *Ciba Foundation Symposium - Steroid Hormones and Enzymes (Book II of Colloquia on Endocrinology)* 123–146 (John Wiley & Sons, Ltd, 1957). doi:10.1002/9780470715239.ch8.
149. Hess, S. G., Diachishin, A. N. & Falco, P. D. Bactericidal Effects of Sewage Chlorination: II. Theoretical Aspects. *Sewage and Industrial Wastes* 25, 909–917 (1953).
150. Hunt, N. K. & Mariñas, B. J. Kinetics of Escherichia coli inactivation with ozone. *Water Research* 31, 1355–1362 (1997).
151. Collins, H. F., Selleck, R. E. & White, G. C. Problems in Obtaining Adequate Sewage Disinfection. *Journal of the Sanitary Engineering Division* 97, 549–562 (1971).
152. Pharma-Zentrale GmbH. Mutaflor. https://www.pharma-zentrale.de/contact.html (2018).
153. Berman, D., Rice, E. W. & Hoff, J. C. Inactivation of particle-associated coliforms by chlorine and monochloramine. *Appl. Environ. Microbiol.* 54, 507–512 (1988).

154. Petterson, S. R. & Stenström, T. A. Quantification of pathogen inactivation efficacy by free chlorine disinfection of drinking water for QMRA. *J Water Health* 13, 625–644 (2015).
155. Wattie, E. & Butterfield, C. T. Relative Resistance of Escherichia coli and Eberthella typhosa to Chlorine and Chloramines. *Public Health Reports (1896-1970)* 59, 1661–1671 (1944).
156. Chauret, C., Smith, C. & Baribeau, H. Inactivation of Nitrosomonas europaea and pathogenic Escherichia coli by chlorine and monochloramine. *J Water Health* 6, 315–322 (2008).
157. Lund, V. Evaluation of E. coli as an indicator for the presence of Campylobacter jejuni and Yersinia enterocolitica in chlorinated and untreated oligotrophic lake water. *Water Research* 30, 1528–1534 (1996).
158. Rice, E. W., Clark, R. M. & Johnson, C. H. Chlorine Inactivation of Escherichia coli O157:H7. *Emerg Infect Dis* 5, 461–463 (1999).
159. Haas, C. N., Keralius, M. G., Brncich, D. M. & Zapkin, M. A. Alteration of chemical and disinfectant properties of hypochlorite by sodium, potassium, and lithium. *Environmental Science & Technology* 20, 822–826 (1986).
160. Masjoudi, M., Mohseni, M. & Bolton, J. R. Sensitivity of Bacteria, Protozoa, Viruses, and Other Microorganisms to Ultraviolet Radiation. *J. RES. NATL. INST. STAN.* 126, 126021 (2021).
161. Beck, S. E. *et al.* Evaluating UV-C LED disinfection performance and investigating potential dual-wavelength synergy. *Water Research* 109, 207–216 (2017).
162. Otaki, M., Okuda, A., Tajima, K. & Iwasaki, T. Inactivation differences of microorganisms by low pressure UV and pulsed xenon lamps. *Progress in Water Technologyy* 13, (2003).
163. Yang, C., Sun, W. & Ao, X. Using mRNA to investigate the effect of low-pressure ultraviolet disinfection on the viability of E. coli. *Front. Environ. Sci. Eng.* 13, 26 (2019).
164. Tosa, K. & Hirata, T. Photoreactivation of enterohemorrhagic Escherichia coli following UV disinfection. *Water Research* 33, 361–366 (1999).
165. Green, A. *et al.* Inactivation of Escherichia coli, Listeria and Salmonella by single and multiple wavelength ultraviolet-light emitting diodes. *Innovative Food Science & Emerging Technologies* 47, 353–361 (2018).
166. McLaren, A. D. & Shugar, D. *Photochemistry of Proteins and Nucleic Acids.* (Pergamon Press, 1964).
167. Kashimada, K., Kamiko, N., Yamamoto, K. & Ohgaki, S. Assessment of photoreactivation following ultraviolet light disinfection. *Water Science and Technology* 33, 261–269 (1996).

168. Sommer, R., Lhotsky, M., Haider, T. & Cabaj, A. UV inactivation, liquid-holding recovery, and photoreactivation of Escherichia coli O157 and other pathogenic Escherichia coli strains in water. *J Food Prot* 63, 1015–1020 (2000).
169. Zimmer, J. L. & Slawson, R. M. Potential Repair of Escherichia coli DNA following Exposure to UV Radiation from Both Medium- and Low-Pressure UV Sources Used in Drinking Water Treatment. *Applied and Environmental Microbiology* 68, 3293–3299 (2002).
170. Ma, D., Weir, M. H. & Hull, N. M. Fluence-based QMRA model for bacterial photorepair and regrowth in drinking water after decentralized UV disinfection. *Water Research* 231, 119612 (2023).
171. Fewtrell, L. *et al.* Water, sanitation, and hygiene interventions to reduce diarrhoea in less developed countries: a systematic review and meta-analysis. *The Lancet Infectious Diseases* 5, 42–52 (2005).
172. Brown, J., Chai, R., Wang, A. & Sobsey, M. D. Microbiological Effectiveness of Mineral Pot Filters in Cambodia. *Environ. Sci. Technol.* 46, 12055–12061 (2012).
173. van Halem, D., Heijman, S. G. J., Soppe, A. I. A., van Dijk, J. C. & Amy, G. L. Ceramic silver-impregnated pot filters for household drinking water treatment in developing countries: material characterization and performance study. *Water Science & Technology: Water Supply* 7, 9–17 (2007).
174. Clasen, T. & Menon, S. Microbiological performance of common water treatment devices for household use in India. *International Journal of Environmental Health Research* 17, 83–93 (2007).
175. Gupta, S. *et al.* Household Production of Ceramic Water Filters in Western Rajasthan, India. *International Journal for Service Learning in Engineering, Humanitarian Engineering and Social Entrepreneurship* 13, 53–66 (2018).
176. Clasen, T. F., Brown, J., Collin, S., Suntura, O. & Cairncross, S. Reducing diarrhea through the use of household-based ceramic water filters: A randomized, controlled trial in rural Bolivia. *Am. J. Trop. Med. Hyg.* 70, 651–657 (2004).
177. Oyanedel-Craver, V. A. & Smith, J. A. Sustainable Colloidal-Silver-Impregnated Ceramic Filter for Point-of-Use Water Treatment. *Environ. Sci. Technol.* 42, 927–933 (2008).
178. Hagan, J. M. *et al.* Resource Development International - Cambodia, Ceramic Water Filter Handbook. (2013).
179. van Halem, D., van der Laan, H., Heijman, S. G. J., van Dijk, J. C. & Amy, G. L. Assessing the sustainability of the silver-impregnated ceramic pot filter for low-cost household drinking water treatment. *Physics and Chemistry of the Earth, Parts A/B/C* 34, 36–42 (2009).
180. Erhuanga, E. A. *et al.* Manufacturing a Ceramic Water Filter Press for Use in Nigeria. *Design Engineering and Manufacturing* (2020) doi:10.5772/intechopen.91378.

181. Brown, J. & Sobsey, M. *Independent Appraisal of Ceramic Water Filtration Interventions in Cambodia: Final Report.* (2006).
182. Johnson, S. M., Peletz, R. & Murcott, S. Results from household ceramic filter evaluation in northern Ghana. (2008).
183. Albert, J., Luoto, J. & Levine, D. End-User Preferences for and Performance of Competing POU Water Treatment Technologies among the Rural Poor of Kenya. *Environ. Sci. Technol.* 44, 4426–4432 (2010).
184. Lemons, A., Branz, A., Kimirei, M., Hawkins, T. & Lantagne, D. Assessment of the quality, effectiveness, and acceptability of ceramic water filters in Tanzania. *Journal of Water, Sanitation and Hygiene for Development* 6, 195–204 (2016).
185. Roberts, M. Field test of a silver-impregnated ceramic water filter. in (2004).
186. Levy, K. Invited Perspective: Environmental Health Interventions Are Only as Good as Their Adoption. *Environ. Health Perspect.* 131, 011303–01 (2023).
187. Muhammad Nur, Sinha Rajib, Krishnan E. Radha, & Patterson Craig L. Ceramic Filter for Small System Drinking Water Treatment: Evaluation of Membrane Pore Size and Importance of Integrity Monitoring. *Journal of Environmental Engineering* 135, 1181–1191 (2009).
188. Bain, R. *et al.* A Summary Catalogue of Microbial Drinking Water Tests for Low and Medium Resource Settings. *International Journal of Environmental Research and Public Health* 9, 1609–1625 (2012).
189. Lee, J.-K. Surface Charge Effects on Particulate Retention by Microporous Membrane Filters in Liquid Filtration. *Environ. Eng. Res.* 3, 97–104 (1998).
190. Nissui Pharmaceutical Co., Ltd. Compact Dry 'Nissui' for Coliform and E. coli. (2009).
191. Champagne, C. P., Ross, R. P., Saarela, M., Hansen, K. F. & Charalampopoulos, D. Recommendations for the viability assessment of probiotics as concentrated cultures and in food matrices. *International Journal of Food Microbiology* 149, 185–193 (2011).
192. Envirogard Products Ltd. SM4 Gravity Water Filter Assembly and Operating Instructions. (2018).
193. Malvern Panalytical. Zetasizer nano series user manual. (2013).
194. Ghasemi, A. & Zahediasl, S. Normality Tests for Statistical Analysis: A Guide for Non-Statisticians. *Int J Endocrinol Metab* 10, 486–489 (2012).
195. Malvern Panalytical. Dynamic Light Scattering (DLS) - Definition & Terms. (2023).
196. Malvern Panalytical. Size quality report for the Zetasizer Nano. (2022).
197. Malvern Panalytical. What Are Number Fluctuations? https://www.malvernpanalytical.com/en/learn/knowledge-center/faqs/faq160715numberfluctuations (2023).

198. Malvern Panalytical. The zeta quality factor in the Zetasizer. https://www.malvernpanalytical.com/en/learn/knowledge-center/technical-notes/tn111214electrophoreticmobility (2022).
199. Venis, R. A. & Basu, O. D. Mechanisms and efficacy of disinfection in ceramic water filters: A critical review. *Critical Reviews in Environmental Science and Technology* 51, 2934–2974 (2021).
200. Bloem. Silver Impregnated Ceramic Water Filter: Flow Rate Versus the REmoval Efficiency of Pathogens. (Delft University of Technology, 2008).
201. Canales, R. A., Wilson, A. M., Pearce-Walker, J. I., Verhougstraete, M. P. & Reynolds, K. A. Methods for Handling Left-Censored Data in Quantitative Microbial Risk Assessment. *Applied and Environmental Microbiology* 84, e01203-18 (2018).
202. Howard, G. *et al.* Domestic water quantity, service level and health, second edition. (2020).
203. Soppe, A. I. A. *et al.* Critical parameters in the production of ceramic pot filters for household water treatment in developing countries. *J Water Health* 13, 587–599 (2015).
204. Youmoue, M. *et al.* Design of ceramic filters using Clay/Sawdust composites: Effect of pore network on the hydraulic permeability. *Ceramics International* 43, 4496–4507 (2017).
205. Lantagne, D. *et al.* Effect of production variables on microbiological removal in locally-produced ceramic filters for household water treatment. *International Journal of Environmental Health Research* 20, 171–187 (2010).
206. Pokharel, H., Shepard, Z. & Oyanedel-Craver, V. Assessing Flow Rate and Nominal Pore Diameter as Parameters for Predicting the Removal of Microorganisms by Ceramic Water Filters. *ACS EST Eng.* 1, 543–550 (2021).
207. White, E. P. *et al.* Assessment of Flow Rate as a Quality Control Test for Ceramic Pot Filters. in *Floods, Droughts and Ecosystems* (2015).
208. National Research Council (USA). Correlates of Smallest Sizes for Microorganisms. in *National Research Council (US) Steering Group for the Workshop on Size Limits of Very Small Microorganisms* (National Academies Press (US), 1999).
209. Cesar, S. & Huang, K. C. Thinking big: the tunability of bacterial cell size. *FEMS Microbiol Rev* 41, 672–678 (2017).
210. Woldringh, C. L., Grover, N. B., Rosenberger, R. F. & Zaritsky, A. Dimensional rearrangement of rod-shaped bacteria following nutritional shift-up. II. Experiments with Escherichia coliBr. *Journal of Theoretical Biology* 86, 441–454 (1980).
211. Taheri-Araghi, S. *et al.* Cell-Size Control and Homeostasis in Bacteria. *Current Biology* 25, 385–391 (2015).
212. Athale, C. A. & Chaudhari, H. Population length variability and nucleoid numbers in Escherichia coli. *Bioinformatics* 27, 2944–2948 (2011).

213. Yoshida, M. *et al.* Directed evolution of cell size in Escherichia coli. *BMC Evolutionary Biology* 14, 257 (2014).
214. Schwegmann, H., Feitz, A. J. & Frimmel, F. H. Influence of the zeta potential on the sorption and toxicity of iron oxide nanoparticles on S. cerevisiae and E. coli. *Journal of Colloid and Interface Science* 347, 43–48 (2010).
215. Halder, S. *et al.* Alteration of Zeta potential and membrane permeability in bacteria: a study with cationic agents. *SpringerPlus* 4, 672 (2015).
216. Kłodzińska, E. *et al.* Effect of zeta potential value on bacterial behavior during electrophoretic separation. *ELECTROPHORESIS* 31, 1590–1596 (2010).
217. Novak, S. *et al.* Electrophoretic deposition as a tool for separation of protein inclusion bodies from host bacteria in suspension. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* 340, 155–160 (2009).
218. Kim, J. K. & Harrison, M. A. Surrogate selection for Escherichia coli O157:H7 based on cryotolerance and attachment to romaine lettuce. *J Food Prot* 72, 1385–1391 (2009).
219. Lytle, D. A., Rice, E. W., Johnson, C. H. & Fox, K. R. Electrophoretic Mobilities of Escherichia coli O157:H7 and Wild-Type Escherichia coliStrains. *Applied and Environmental Microbiology* 65, 3222–3225 (1999).
220. Soni, K. A., Balasubramanian, A. K., Beskok, A. & Pillai, S. D. Zeta Potential of Selected Bacteria in Drinking Water When Dead, Starved, or Exposed to Minimal and Rich Culture Media. *Curr Microbiol* 56, 93–97 (2008).
221. Brown, J. & Sobsey, M. D. Ceramic media amended with metal oxide for the capture of viruses in drinking water. *Environmental Technology* 30, 379–391 (2009).
222. Clasen, T., Nadakatti, S. & Menon, S. Microbiological performance of a water treatment unit designed for household use in developing countries. *Tropical Medicine & International Health* 11, 1399–1405 (2006).
223. Jamart, J. Statistical Tests in Medical Research. *Acta Oncologica* 31, 723–727 (1992).
224. Carley, S. & Lecky, F. Statistical consideration for research. *Emerg Med J* 20, 258–262 (2003).
225. LeFort, S. M. The Statistical versus Clinical Significance Debate. *Image: the Journal of Nursing Scholarship* 25, 57–62 (1993).
226. Motlagh, A. M. & Yang, Z. Detection and occurrence of indicator organisms and pathogens. *Water Environ Res* 91, 1402–1408 (2019).
227. Yates, M. V. Classical Indicators in the 21st Century—Far and Beyond the Coliform. *Water Environment Research* 79, 279–286 (2007).
228. Lucier, K. J., Dickson-Anderson, S. E. & Schuster-Wallace, C. J. Effectiveness of silver and copper infused ceramic drinking water filters in reducing microbiological

contaminants. *Journal of Water Supply: Research and Technology - Aqua* 66, 528–536 (2017).

229. Rayner, J. & Lantagne, D. Investigations on Household Filtration in Haiti: Final Report. (2014).
230. Murphy, McBean, E. A. & Farahbakhsh, K. A critical evaluation of two point-of-use water treatment technologies: can they provide water that meets WHO drinking water guidelines? *J Water Health* 8, 611–630 (2010).
231. Murphy, Sampson, M., Farahbakhsh, K. & McBean, E. Microbial and chemical assessment of ceramic and BioSand water filters in rural Cambodia. *Water Science and Technology: Water Supply* 10, 286–295 (2010).
232. Schweitzer, R. Community and Household Management Strategies for Water Supply and Treatment in Rural and Peri-urban Areas in the Developing World. (2013).
233. Stubbé, S. M. L., Pelgrim-Adams, A., Szántó, G. L. & Halem, D. van. Household water treatment and safe storage – effectiveness and economics. *Drinking Water Engineering and Science* 9, 9–18 (2016).
234. Lantagne, D. *Investigation of the Potters for Peace Colloidal Silver Impregnated Ceramic Filter.* http://www.bionica.info/Biblioteca/lantagne2001environmentalinvestigationnic.pdf (2001).
235. Ren, D., Colosi, L. M. & Smith, J. A. Evaluating the Sustainability of Ceramic Filters for Point-of-Use Drinking Water Treatment. *Environ. Sci. Technol.* 47, 11206–11213 (2013).
236. Heylen, C. *et al.* Laboratory Efficacy of Locally Available Backwashing Methods at Removing Fouling in Hollow-Fiber Membrane Filters Used for Household Water Treatment. *Membranes* 11, 375 (2021).
237. Holding, S. *et al.* Acceptability, effectiveness, and fouling of PointOne membrane filters distributed in South Sudan. *Journal of Water, Sanitation and Hygiene for Development* 9, 247–257 (2019).
238. Akowanou, A. V. O. *et al.* 3D-printed clay-based ceramic water filters for point-of-use water treatment applications. *Prog Addit Manuf* 4, 315–321 (2019).
239. Guo, W., Ngo, H.-H. & Li, J. A mini-review on membrane fouling. *Bioresource Technology* 122, 27–34 (2012).
240. Mahlangu, O. T., Mubiayi, M. P. & Mamba, B. B. A facile approach for the synthesis of ceramic filters for methyl orange, chromium and lead removal from water. *Physics and Chemistry of the Earth, Parts A/B/C* 129, 103368 (2023).
241. Ehrlich, H. L. Are gram-positive bacteria capable of electron transfer across their cell wall without an externally available electron shuttle? *Geobiology* 6, 220–224 (2008).
242. Health Canada. Guidelines for Canadian Drinking Water Quality: Guideline Technical Document, Enteric Protozoa: Giardia and Cryptosporidium. *aem* https://www.canada.ca/en/health-canada/services/environmental-workplace-

health/reports-publications/water-quality/enteric-protozoa-giardia-cryptosporidium.html (2019).

243. WHO. Risk Assessment of Cryptosporidium in Drinking Water. (2009).
244. Elliott, J. G., Taylor-Edmonds, L. & Andrews, R. C. Quantitative microbial risk assessments for drinking water facilities: evaluation of a range of treatment strategies. *Environmental Science: Water Research & Technology* 5, 1943–1955 (2019).
245. USEPA. Long Term 2 Enhanced Surface Water Treatment Rule Toolbox Guidance Manual. (2010).
246. USEPA. Disinfection Profiling and Benchmarking: Technical Guidance. (2020).
247. Hendricks, D. W. *et al.* Filtration Removals of Microorganisms and Particles. *Journal of Environmental Engineering* 131, 1617–1769 (2005).
248. USEPA. Small Drinking Water Systems Handbook: A Guide to 'Packaged' Filtration and Disinfection Technologies with Remote Monitoring and Control Tools. (2003).
249. USEPA. Method 1623: Cryptosporidium and Giardia in Water by Filtration/IMS/FA. (2005).
250. Nieminski, E. C., Schaefer, F. W. & Ongerth, J. E. Comparison of two methods for detection of Giardia cysts and Cryptosporidium oocysts in water. *Appl. Environ. Microbiol.* 61, 1714–1719 (1995).
251. Clancy, J. L., Bukhari, Z., McCuin, R. M., Matheson, Z. & Fricker, C. R. USEPA method 1622. *Journal AWWA* 91, 60–68 (1999).
252. Hsu, B., Huang, C., Hsu, Y., Jiang, G. & Hsu, C. lilian. Evaluation of two concentration methods for detecting Giardia and Cryptosporidium in water. *Water Research* 35, 419–424 (2001).
253. Quintero-Betancourt, W., Peele, E. R. & Rose, J. B. Cryptosporidium parvum and Cyclospora cayetanensis: a review of laboratory methods for detection of these waterborne parasites. *Journal of Microbiological Methods* 49, 209–224 (2002).
254. LeChevallier, M. W. & Norton, W. D. Giardia and Cryptosporidium in raw and finished water. *Journal AWWA* 87, 54–68 (1995).
255. Medema, G. J. *et al.* Quantitative risk assessment of Cryptosporidium in surface water treatment. *Water Sci Technol* 47, 241–247 (2003).
256. Muhammad, N. *et al.* Evaluating surrogates for Cryptosporidium removal in point-of-use systems. *American Water Works Association. Journal; Denver* 100, 98-107,12 (2008).
257. Li, S. Y. *et al.* Reliability of surrogates for determining Cryptosporidium removal. *Journal AWWA* 89, 90–99 (1997).
258. Hijnen, W. A. M., Houtepen, F. A. P., van der Speld, W. M. H. & van der Kooij, D. Spores of sulphite-reducing clostridia: a surrogate parameter for assessing the effects of water treatment processes on protozoan (oo)cysts? in 115–126 (1997).

259. Jakubowski, W. *et al.* Environmental methods for Cryptosporidium. *Journal (American Water Works Association)* 88, 107–121 (1996).
260. Sun, Z., Li, M., Li, W. & Qiang, Z. A review of the fluence determination methods for UV reactors: Ensuring the reliability of UV disinfection. *Chemosphere* 286, 131488 (2022).
261. Mercado, L. F. P. On-farm filtration technology for pathogen reduction. (Swedish University of Agricultural Sciences, 2017).
262. Beuchat, L. R. Chapter 22: Media for detecting and enumerating yeasts and moulds. in *Progress in Industrial Microbiology* (eds. Corry, J. E. L., Curtis, G. D. W. & Baird, R. M.) vol. 37 369–385 (Elsevier, 2003).
263. International Organization for Standardization. ISO 21527-1:2008. (2008).
264. Tournas, V., Stack, M., Mislivec, P. B. & Koch, H. A. Chapter 18: Yeasts. Molds, and Mycotoxins. in *Yeasts. Molds, and Mycotoxins* (AOAC Internationa, 1998).
265. Bird, P. *et al.* Evaluation of the 3M™ Petrifilm™ Rapid Yeast and Mold Count Plate for the Enumeration of Yeast and Mold in Food: Collaborative Study, First Action 2014.05. *Journal of AOAC INTERNATIONAL* 98, 767–783 (2015).
266. Association of Official Agricultural Chemists. Simplate Yeast and Mold-Color Indicator (Y&M-CI) Method. (2005).
267. Hernández, A. *et al.* Spoilage yeasts: What are the sources of contamination of foods and beverages? *International Journal of Food Microbiology* 286, 98–110 (2018).
268. Health Canada. Guidelines for Canadian Drinking Water Quality: Guideline Technical Document – Turbidity. (2012).
269. Rose, J. B., Gerba, C. P. & Jakubowski, W. Survey of potable water supplies for Cryptosporidium and Giardia. *Environmental Science & Technology* 25, 1393–1400 (1991).
270. LeChevallier, M. W. & Norton, W. D. Examining Relationships Between Particle Counts and Giardia, Cryptosporidium, and Turbidity. *Journal AWWA* 84, 54–60 (1992).
271. Monis, P., Lau, M., Harris, M., Cook, D. & Drikas, M. Risk-based management of drinking water safety in Australia: Implementation of health based targets to determine water treatment requirements and identification of pathogen surrogates for validation of conventional filtration. *Food and Waterborne Parasitology* 8–9, 64–74 (2017).
272. O'Shaughnessy, P. T., Barsotti, M. G., Fay, J. W. & Tighe, S. W. Evaluating particle counters. *Journal AWWA* 89, 60–70 (1997).
273. Morse, A., Wilson, R., Hull, A. & Simms, J. Monitoring for Cryptosporidium risk: do particle counters help? *Water Supply* 2, 171–180 (2002).

274. Hamilton, P. D., Standen, G., Evans, A. D., Doyle, J. D. & Parsons, S. A. The Use of Particle-Size Counting in Minimising Cryptosporidium Risk at a Groundwater Supply Works. *Water and Environment Journal* 14, 377–384 (2000).
275. Pang, L. *et al.* Biotin- and Glycoprotein-Coated Microspheres: Potential Surrogates for Studying Filtration of Cryptosporidium parvum in Porous Media. *Environ. Sci. Technol.* 46, 11779–11787 (2012).
276. Pang, L. *et al.* Cryptosporidium surrogate removal in five commonly used point-of-use domestic filters. *Journal of Water Process Engineering* 44, 102390 (2021).
277. Dai, X. & Hozalski, R. M. Evaluation of Microspheres as Surrogates for Cryptosporidium parvum Oocysts in Filtration Experiments. *Environ. Sci. Technol.* 37, 1037–1042 (2003).
278. Emelko, M. B., Huck, P. M. & Douglas, I. P. Cryptosporidium and microsphere removal during late in-cycle filtration. *Journal AWWA* 95, 173–182 (2003).
279. Emelko, M. B. & Huck, P. M. Microspheres as Surrogates for Cryptosporidium Filtration. *Journal AWWA* 96, 94–105 (2004).
280. Kim, H.-S., Shikiya, J., Akiba, M. & Kunikane, S. Examination of monodispersed artificial particles similar to C. parvum oocysts in size as the removal surrogate of Cryptosporidium. *Journal of Water Supply: Research and Technology - Aqua* 53, 51–59 (2004).
281. Liu, L. *et al.* Removal of Cryptosporidium surrogates in drinking water direct filtration. *Colloids and Surfaces B: Biointerfaces* 181, 499–505 (2019).
282. Amburgey, J. E. *et al.* Comparison of Conventional and Biological Filter Performance for Cryptosporidium and microsphere removal. *Journal AWWA* 97, 77–91 (2005).
283. Blatchley, E. R. *et al.* Validation of large-scale, monochromatic UV disinfection systems for drinking water using dyed microspheres. *Water Research* 42, 677–688 (2008).
284. Shen, C. *et al.* Validation of medium-pressure UV disinfection reactors by Lagrangian actinometry using dyed microspheres. *Water Research* 43, 1370–1380 (2009).
285. Zhao, X., Alpert, S. M. & Ducoste, J. J. Assessing the Impact of Upstream Hydraulics on the Dose Distribution of Ultraviolet Reactors Using Fluorescence Microspheres and Computational Fluid Dynamics. *Environmental Engineering Science* 26, 947–959 (2009).
286. Baeza, C. & Ducoste, J. A non-biological surrogate for sequential disinfection processes. *Water Research* 38, 3400–3410 (2004).
287. Richards, B. & Ducoste, J. Application of non-biological surrogates for analysis of sequential disinfection continuous flow systems. *Journal of Water Supply: Research and Technology-Aqua* 57, 225–238 (2008).

288. Chung-Fan, C., Torres-Lugo, M., Marinas, B. J. & Adams, J. Q. Nonbiological surrogate indicators for assessing ozone disinfection. *American Water Works Association. Journal; Denver* 89, 54 (1997).
289. Mariñas, B. J., Rennecker, J. L., Teefy, S. & Rice, E. W. Assessing ozone disinfection with nonbiological surrogates. *Journal - AWWA* 91, 79–89 (1999).
290. Tang, G. *et al.* Modeling Cryptosporidium parvum Oocyst Inactivation and Bromate Formation in a Full-Scale Ozone Contactor. *Environ. Sci. Technol.* 39, 9343–9350 (2005).
291. Dugan, N. R., Fox, K. R., Owens, J. H. & Miltner, R. J. Controlling Cryptosporidium oocysts Using Conventional Treatment. *Journal AWWA* 93, 64–76 (2001).
292. Hijnen, W. A., Willemsen-Zwaagstra, J., Hiemstra, P., Medema, G. J. & van der Kooij, D. Removal of sulphite-reducing clostridia spores by full-scale water treatment processes as a surrogate for protozoan (oo)cysts removal. *Water Sci Technol* 41, 165–171 (2000).
293. Payment, P. & Franco, E. Clostridium perfringens and somatic coliphages as indicators of the efficiency of drinking water treatment for viruses and protozoan cysts. *Appl. Environ. Microbiol.* 59, 2418–2424 (1993).
294. Brown, R. A. & Cornwell, D. A. Using spore removal to monitor plant performance for Cryptosporidium removal. *Journal AWWA* 99, 95–109 (2007).
295. Cornwell, D. A., MaCphee, M. J., Brown, R. A. & Via, S. H. Demonstrating Cryptosporidium Removal using spore monitoring at lime-softening plants. *Journal AWWA* 95, 124–133 (2003).
296. Hijnen, W. A. M. *et al.* Enumeration of faecal indicator bacteria in large water volumes using on site membrane filtration to assess water treatment efficiency. *Water Research* 34, 1659–1665 (2000).
297. Hijnen, W. A. M., Beerendonk, E. F. & Medema, G. J. Inactivation credit of UV radiation for viruses, bacteria and protozoan (oo)cysts in water: A review. *Water Research* 40, 3–22 (2006).
298. Rattanakul, S. & Oguma, K. Inactivation kinetics and efficiencies of UV-LEDs against Pseudomonas aeruginosa, Legionella pneumophila, and surrogate microorganisms. *Water Research* 130, 31–37 (2018).
299. Facile, N., Barbeau, B., Prévost, M. & Koudjonou, B. Evaluating bacterial aerobic spores as a surrogate for Giardia and Cryptosporidium inactivation by ozone. *Water Research* 34, 3238–3246 (2000).
300. Headd, B. & Bradford, S. A. Use of aerobic spores as a surrogate for cryptosporidium oocysts in drinking water supplies. *Water Research* 90, 185–202 (2016).
301. Venczel, L. V., Arrowood, M., Hurd, M. & Sobsey, M. D. Inactivation of Cryptosporidium parvum oocysts and Clostridium perfringens spores by a mixed-

oxidant disinfectant and by free chlorine. *Appl. Environ. Microbiol.* 63, 1598–1601 (1997).

302. Chauret, C. P., Radziminski, C. Z., Lepuil, M., Creason, R. & Andrews, R. C. Chlorine Dioxide Inactivation ofCryptosporidium parvum Oocysts and Bacterial Spore Indicators. *Appl. Environ. Microbiol.* 67, 2993–3001 (2001).
303. Akiba, M., Kunikane, S., Kim, H.-S. & Kitazawa, H. Algae as surrogate indices for the removal of Cryptosporidium oocysts by direct filtration. *Water Science and Technology: Water Supply* 2, 73–80 (2002).
304. Kim, H.-S., Kobayashi, Y., Akiba, M. & Kunikane, S. Evaluation of Scenedesmus quadricauda as a surrogate of Cryptosporidium oocysts removal in direct filtration. *Water Science and Technology: Water Supply* 2, 395–402 (2002).
305. Hijnen, W. A. M. *et al.* Removal and fate of Cryptosporidium parvum, Clostridium perfringens and small-sized centric diatoms (Stephanodiscus hantzschii) in slow sand filters. *Water Research* 41, 2151–2162 (2007).
306. Higgins, J. *et al. Cochrane Handbook for Systematic Reviews of Interventions.* (Cochrane, 2023).
307. Moher, D., Liberati, A., Tetzlaff, J. & Altman, D. G. Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. *PLOS Medicine* 6, e1000097 (2009).
308. Page, M. J. *et al.* The PRISMA 2020 statement: An updated guideline for reporting systematic reviews. *International Journal of Surgery* 88, 105906 (2021).
309. Pieper, D. & Rombey, T. Where to prospectively register a systematic review. *Systematic Reviews* 11, 8 (2022).
310. Azar Daryany, M. K., Massudi, R. & Hosseini, M. Photoinactivation of Escherichia coli and Saccharomyces cerevisiae Suspended in Phosphate-Buffered Saline-A Using 266- and 355-nm Pulsed Ultraviolet Light. *Curr Microbiol* 56, 423–428 (2008).
311. Buschini, A., Carboni, P., Furlini, M., Poli, P. & Rossi, C. Sodium hypochlorite-, chlorine dioxide- and peracetic acid-induced genotoxicity detected by the Comet assay and Saccharomyces cerevisiae D7 tests. *Mutagenesis* 19, 157–162 (2004).
312. Del Carratore, R. *et al.* Cell cycle and morphological alterations as indicative of apoptosis promoted by UV irradiation in S. cerevisiae. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis* 513, 183–191 (2002).
313. Dubeau, H. & Chung, Y. S. Ozone response in wild type and radiation-sensitive mutants of Saccharomyces cerevisiae. *Molec. gen. Genet.* 176, 393–398 (1979).
314. Hays, H., Elliker, P. R. & Sandine, W. E. Microbial Destruction by Low Concentrations of Hypochlorite and Iodophor Germicides in Alkaline and Acidified Water. *Appl. Environ. Microbiol.* 15, 575–581 (1967).
315. Schenk, M., Raffellini, S., Guerrero, S., Blanco, G. A. & Alzamora, S. M. Inactivation of Escherichia coli, Listeria innocua and Saccharomyces cerevisiae by UV-C light:

Study of cell injury by flow cytometry. *LWT - Food Science and Technology* 44, 191–198 (2011).

316. Sommer, R., Haider, T., Cabaj, A., Heidenreich, E. & Kundi, M. Increased inactivation of Saccharomyces cerevisiae by protraction of UV irradiation. *Appl. Environ. Microbiol.* 62, 1977–1983 (1996).
317. Tsukamoto, I., Constantinoiu, E., Furuta, M., Nishimura, R. & Maeda, Y. Inactivation effect of sonication and chlorination on Saccharomyces cerevisiae. Calorimetric Analysis. *Ultrasonics Sonochemistry* 11, 167–172 (2004).
318. Watanabe, M. *et al.* Inactivation Effects of UV Irradiation and Ozone Treatment on the Yeast and the Mold in Mineral Water. *Journal of Food Protection* 73, 1537–1542 (2010).
319. Nardini & Wang. The Yale MeSH Analyzer. http://mesh.med.yale.edu/help#whatis (2020).
320. Stern, C., Jordan, Z. & McArthur, A. Developing the Review Question and Inclusion Criteria. *AJN The American Journal of Nursing* 114, 53–56 (2014).
321. Pedder, H., Sarri, G., Keeney, E., Nunes, V. & Dias, S. Data extraction for complex meta-analysis (DECiMAL) guide. *Systematic Reviews* 5, 212 (2016).
322. Rohatgi, A. WebPlotDigitizer. https://apps.automeris.io/wpd/ (2020).
323. Bain, R. *et al.* Fecal Contamination of Drinking-Water in Low- and Middle-Income Countries: A Systematic Review and Meta-Analysis. *PLoS Med* 11, (2014).
324. Williams, A. R. *et al.* A Systematic Review and Meta-Analysis of Fecal Contamination and Inadequate Treatment of Packaged Water. *PLOS ONE* 10, e0140899 (2015).
325. Ramesh, A., Blanchet, K., Ensink, J. H. J. & Roberts, B. Evidence on the Effectiveness of Water, Sanitation, and Hygiene (WASH) Interventions on Health Outcomes in Humanitarian Crises: A Systematic Review. *PLOS ONE* 10, e0124688 (2015).
326. Moher, D., Schulz, K. F. & Altman, D. G. The CONSORT statement: revised recommendations for improving the quality of reports of parallel-group randomised trials. *Clin Oral Invest* 7, 2–7 (2003).
327. von Elm, E. *et al.* The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement: Guidelines for Reporting Observational Studies. *Annals of Internal Medicine* 147, 573–577 (2007).
328. Borenstein, M., Hedges, L., Higgins, J. & Rothstein, H. *Introduction to Meta-Analysis*. (John Wiley & Sons, Ltd, 2009). doi:10.1002/9780470743386.
329. USEPA. Ultaviolet Disinfection Guidance Manual for the Final Long Term 2 Enhanced Surface Water Treatment Rule. (2006).

330. Bisquert, R., Muniz-Calvo, S. & Guillamon, J. M. Protective Role of Intracellular Melatonin Against Oxidative Stress and UV Radiation in Saccharomyces cerevisiae. *FRONTIERS IN MICROBIOLOGY* vol. 9 (2018).
331. Kim, J. K., Petin, V. G. & Tkhabisimova, M. D. Survival and recovery of yeast cells after simultaneous treatment of UV light radiation and heat. *Photochem. Photobiol.* 79, 349–355 (2004).
332. Mijowska, K. *et al.* Preliminary study on the influence of UV-C irradiation on microorganism viability and polyphenol compounds content during winemaking of `Regent' red grape cultivar. *Polish Journal of Chemical Technology* 19, 130–7 (2017).
333. Moustacchi, E. & Enteric, S. Differential 'liquid holding recovery' for the lethal effect and cytoplasmic 'petite' induction by UV light in Saccharomyces cerevisiae. *Mol. Gen. Genet.* 109, 69–83 (1970).
334. Nunes, E., Lorido, L. & Gelos, U. Survival of synchronized diploid yeast after ultraviolet irradiation. *Radiation Research* 63, 474–82 (1975).
335. Parry, E. M. & Parry, J. M. The genetic control of liquid-holding recovery and U.V.-induced repair resistance in the yeast, Saccharomyces cerevisiae. *Int. J. Radiat. Biol. Relat. Stud. Phys. Chem. Med.* 30, 13–24 (1976).
336. Zuk, J., Zaborowska, D. & Swietlińska, Z. Effect of caffeine on recovery from DEB-induced cell inactivation in UV-sensitive mutants of Saccharomyces cerevisiae. *Mutat. Res.* 33, 173–178 (1975).
337. Zuk, J., Zaborowska, D. & Swietlińska, Z. Comparison of sensitivity and liquid holding recovery in rad mutants of Saccharomyces cerevisiae inactivated by UV and DEB. *Mol. Gen. Genet.* 166, 91–96 (1978).
338. Waters, R. & Parry, J. M. A comparative study of the effects of UV irradiation upon diploid cultures of yeast defective at the rad 3 locus. *Mol. Gen. Genet.* 124, 145–156 (1973).
339. Swietlinska, Z., Zaborowska, D. & Zuk, J. Survival and liquid holding recovery in UV-sensitive strains of Sarrharomyces cerevisiae after treatment with chemical mutagens and radiation. *Microbiologica Polonica* vol. 25 295–305 (1976).
340. Mucka, V., Blaha, P. & Cuba, V. Measurement of growth and survival curves of microorganisms influenced by radiation. *Journal of Radioanalytical and Nuclear Chemistry* 286, 603–10 (2010).
341. Parry, J. M. The genetic effects of liquid holding recovery in ultra violet light sensitive mutants of yeast. *Mol. Gen. Genet.* 111, 51–60 (1971).
342. Parry, J. M. A quantitative analysis of 'negative liquid holding', in some UV sensitive mutants of yeast. *Mol. Gen. Genet.* 118, 33–43 (1972).
343. Parry, J. & Parry, E. Genetic implications of UV light exposure and liquid-holding post-treatment in yeast Saccharoyces cerevisiae. *Genetical Research* vol. 19 1- (1972).

344. Pirnie, M. & HDR Engineering. *Guidance Manual for Compliance with the Filtration and Disinfection Requirements for Public Water Systems Using Surface Water Sources*. (American Water Works Association, 1991).
345. Qian, S. S. *et al.* Ultraviolet light inactivation of protozoa in drinking water: a Bayesian meta-analysis. *Water Research* 38, 317–326 (2004).
346. Haas, C. N., Severin, B. F. & Roy, D. Field observations on the occurrence of new indicators of disinfection efficiency. *Water Research* 19, 323–329 (1985).
347. Engelbrecht, R. S. *et al.* New Microbial Indicators of Disinfection Efficacy. (1977).
348. Haas, C. N. & Karra, S. B. Kinetics of microbial inactivation by chlorine—I Review of results in demand-free systems. *Water Research* 18, 1443–1449 (1984).
349. UN. *Sustainable Development Goals Report.* https://unstats.un.org/sdgs/report/2018 (2018).
350. Kelner, A. Effect of Visible Light on the Recovery of Streptomyces Griseus Conidia from Ultra-violet Irradiation Injury*. *Proceedings of the National Academy of Sciences* 35, 73–79 (1949).
351. Kelner, A. Action Spectra for Photoreactivation of Ultraviolet-irradiated Escherichia coli and Streptomyces griseus. *Journal of General Physiology* 34, 835–852 (1951).
352. Bolton, J. R. & Cotton, C. A. *The Ultraviolet Disinfection Handbook*. (American Water Works Association, 2011).
353. Dadonaite, B. More than half a million children die from diarrhea each year. How do we prevent this? *Our World in Data* https://ourworldindata.org/childhood-diarrheal-diseases (2019).
354. Quick, R. E. *et al.* Diarrhea prevention through household-level water disinfection and safe storage in Zambia. *The American Journal of Tropical Medicine and Hygiene* 66, 584–589 (2002).
355. WHO. Results of the WHO International Scheme to Evaluate Household Water Treatment Technologies. (2016).
356. Ngai, T. K. K., Coff, B., Baker, D. & Lentz, R. Global Review of the Adoption, Use and Performance of the Biosand Filter. in *Progress in Slow Sand and Alternative Biofiltration Processes* (IWA Publishing, 2014).
357. Wright, J., Gundry, S. & Conroy, R. Household drinking water in developing countries: a systematic review of microbiological contamination between source and point-of-use. *Tropical Medicine & International Health* 9, 106–117 (2004).
358. Fleet, G. Spoilage Yeasts. *Critical Reviews in Biotechnology* 12, 1–44 (1992).
359. Bernardes, C., Bernardes, R., Zimmer, C. & Dorea, C. C. A Simple Off-Grid Incubator for Microbiological Water Quality Analysis. *Water* 12, 240 (2020).
360. IDEXX. Colilert - IDEXX US. https://www.idexx.com/en/water/water-products-services/colilert/ (2017).

361. Haas, C., Rose, J. & Gerba, C. *Quantitative Microbial Risk Assessment*. (John Wiley & Sons, Inc., 2014).
362. Murphy, H. M. *et al.* Estimating the burden of acute gastrointestinal illness due to Giardia, Cryptosporidium, Campylobacter, E. coli O157 and norovirus associated with private wells and small water systems in Canada. *Epidemiology & Infection* 144, 1355–1370 (2016).
363. Lucas, B. *Water Quality Inventory of the Nitinat River and San Juan River Watersheds*. https://www2.gov.bc.ca/assets/gov/environment/air-land-water/water/waterquality/monitoring-water-quality/west-coast-wq-docs/wq_nitinat_sanjuan_1997.pdf (1998).
364. Pintar, K. D. M. *et al.* A Comparative Exposure Assessment of Campylobacter in Ontario, Canada. *Risk Analysis* 37, 677–715 (2017).
365. Rosa, G., Huaylinos, M. L., Gil, A., Lanata, C. & Clasen, T. Assessing the Consistency and Microbiological Effectiveness of Household Water Treatment Practices by Urban and Rural Populations Claiming to Treat Their Water at Home: A Case Study in Peru. *PLOS ONE* 9, e114997 (2014).
366. Rosa, G., Kelly, P. & Clasen, T. Consistency of Use and Effectiveness of Household Water Treatment Practices Among Urban and Rural Populations Claiming to Treat Their Drinking Water at Home: A Case Study in Zambia. *The American Journal of Tropical Medicine and Hygiene* 94, 445–455 (2016).
367. Rosa, G. & Clasen, T. Consistency of Use and Effectiveness of Household Water Treatment among Indian Households Claiming to Treat Their Water. *Am J Trop Med Hyg* 97, 259–270 (2017).
368. Medema, G. J., Teunis, P. F. M., Havelaar, A. H. & Haas, C. N. Assessment of the dose-response relationship of Campylobacter jejuni. *International Journal of Food Microbiology* 30, 101–111 (1996).
369. USEPA. *Quantitative Microbial Risk Assessment to Estimate Illness in Freshwater Impacted by Agricultural Animal Sources of Fecal Contamination.* https://www.epa.gov/sites/default/files/2015-11/documents/quantitiave-microbial-risk-fecal.pdf (2010).
370. Health Canada. Guidelines for Canadian Drinking Water Quality: Guideline Technical Document – Enteric Protozoa: Giardia and Cryptosporidium. (2012).
371. Medentech Ltd. Aquatabs | FAQ. https://www.aquatabs.com/faq.php.
372. Pristine Canada. Pristine® Water Purification System 30ml. *Pristine* https://www.pristine.ca/product-page/pristine-water-purification-system-30ml.
373. Grayl USA. 24oz GEOPRESS® Purifier. *Grayl Canada* https://www.grayl.ca/products/geopress-purifier-covert-black.
374. Katadyn Group. Katadyn Base Camp Pro 10L Instruction Manual.
375. Katadyn Group. Katadyn BeFree 0.6L.

376. Katadyn Group. Manual Hiker Pro_USA_en.pdf.
377. Katadyn Group. Manual Vario_en.pdf.
378. Nguyen, C., Pham, H. & Cao, L. Performance on Longevity, Microbial Removal and Chlorine Removal of LifeStraw Flex. (2017).
379. Miniwell Filters. Miniwell L630 Portable Water Filter - Miniwell Filters. https://www.miniwellfilters.com/shop/miniwell-l630-portable-water-filter-straw/.
380. MSR. AutoFlow™ XL 10L Gravity Water Filter for Groups | MSR®. https://www.msrgear.com/ca/water-treatment/filters-and-purifiers/autoflow-xl-gravity-filter-10-l/10944.html.
381. MSR. Guardian™ Purifier Backpacking Water Purifier | MSR®. https://www.msrgear.com/ca/water-treatment/filters-and-purifiers/guardian-purifier/02370.html.
382. MSR. HyperFlow™ Microfilter - Backpacking Water Filter | MSR®. https://www.msrgear.com/ca/water-treatment/filters-and-purifiers/hyperflow-microfilter/56500.html.
383. MSR. MiniWorks® EX Microfilter - Backpacking Water Filter | MSR®. https://www.msrgear.com/ca/water-treatment/filters-and-purifiers/miniworks-ex-microfilter/56425.html.
384. MSR. TrailShot™ Water Filter Personal Water Filter | MSR®. https://www.msrgear.com/ca/water-treatment/filters-and-purifiers/trailshot-pocket-sized-water-filter/09385.html.
385. Platypus. GravityWorks™ Gravity Water Filter System | Platypus®. https://www.platy.com/ca/filtration/gravityworks-water-filter-system-6.0l4.0l/gravityworks.html.
386. Sawyer Products. Micro Squeeze Water Filtration System | Sawyer Products. https://www.sawyer.com/products/micro-squeeze-water-filtration-system.
387. Sawyer Products. MINI Water Filtration System. *Sawyer Products* https://sawyer.com/products/mini-filter/.
388. The Collaboratory & Messiah College. Microbiological testing of the Sawyer Mini Filter. (2013).
389. Sawyer Products. Squeeze Water Filtration System | Sawyer Products. https://www.sawyer.com/products/squeeze-water-filtration-system.
390. Survivor Filter. SURVIVOR FILTER™ PRO X Electric Water Filter. *Survivor Filter* https://www.survivorfilter.com/products/survivor-filter-pro-x-electric-water-filter.
391. Intertek. *Non-Standardized Test Report For: Survivor Filter.* https://cdn.shopify.com/s/files/1/0741/5997/files/102092758COL-003.pdf?12021991132690881172 (2015).

392. HydroBlu. Versa Flow Light-Weight Water Filter. *HydroBlu* https://hydroblu.com/versa-flow-light-weight-water-filter/.
393. Katadyn Group. SPPF-MP-EFG_Steripen Classic 3_en.pdf.
394. Boulware, D. R., Forgey, W. W. & Martin, W. J. Medical risks of wilderness hiking. *The American Journal of Medicine* 114, 288–293 (2003).
395. Dale, A. P. Outbreak of Acute Gastroenteritis Among Rafters and Backpackers in the Backcountry of Grand Canyon National Park, April–June 2022. *MMWR Morb Mortal Wkly Rep* 71, (2022).
396. WHO. *Guidelines for Drinking Water Quality.* http://apps.who.int/iris/bitstream/10665/44584/1/9789241548151_eng.pdf (2011).
397. USEPA. Recreational Water Quality Criteria and Methods. (2012).
398. Petterson, S. R. & Ashbolt, N. J. QMRA and water safety management: review of application in drinking water systems. *J Water Health* 14, 571–589 (2016).
399. Ishaq, S., Sadiq, R., Chhipi-Shrestha, G., Farooq, S. & Hewage, K. Developing an Integrated "Regression-QMRA method" to Predict Public Health Risks of Low Impact Developments (LIDs) for Improved Planning. *Environmental Management* (2022) doi:10.1007/s00267-022-01657-0.
400. The HWTS Network & CAWST. HWTS Network Sanitary Inspection Forms - Draft. https://www.hwts.info/network/sanitary-inspection-forms (2022).
401. WHO, The Aquaya Institute & CAWST. Taking the poop out of water: from global to local, strengthening capacity to test and regulate water treatment technologies. (2022).
402. WHO. *Guidelines for drinking-water quality,.* vol. 3 (1997).
403. Teunis, P. *et al.* A reconsideration of the Campylobacter dose–response relation. *Epidemiology & Infection* 133, 583–592 (2005).
404. Levine, M. M. & Robins-Browne, R. M. Factors That Explain Excretion of Enteric Pathogens by Persons Without Diarrhea. *Clinical Infectious Diseases* 55, S303–S311 (2012).
405. Clasen, T. F. *et al.* Interventions to improve water quality for preventing diarrhoea. *Cochrane Database of Systematic Reviews* (2015) doi:10.1002/14651858.CD004794.pub3.
406. Schmidt, W.-P. & Cairncross, S. Household Water Treatment in Poor Populations: Is There Enough Evidence for Scaling up Now? *Environ. Sci. Technol.* 43, 986–992 (2009).
407. Coughlin, S. S. Recall bias in epidemiologic studies. *Journal of Clinical Epidemiology* 43, 87–91 (1990).
408. Mara D. D. & Feachem R. G. A. Water- and Excreta-Related Diseases: Unitary Environmental Classification. *Journal of Environmental Engineering* 125, 334–339 (1999).

409. Ganesh, A. & Lin, J. Waterborne human pathogenic viruses of public health concern. *International Journal of Environmental Health Research* 23, 544–564 (2013).
410. Fong, T.-T. & Lipp, E. K. Enteric Viruses of Humans and Animals in Aquatic Environments: Health Risks, Detection, and Potential Water Quality Assessment Tools. *Microbiol. Mol. Biol. Rev.* 69, 357–371 (2005).
411. Teunis, P. F. M. *et al.* Norwalk virus: how infectious is it? *J Med Virol* 80, 1468–1476 (2008).
412. Gall, A. M., Mariñas, B. J., Lu, Y. & Shisler, J. L. Waterborne Viruses: A Barrier to Safe Drinking Water. *PLoS Pathog* 11, (2015).
413. Wang, H. & Middelberg, A. P. J. Non-infectious virus-like particles for the validation of membrane integrity and column performance in bioprocessing. *Food and Bioproducts Processing* 98, 327–332 (2016).
414. Cetlin, D. *et al.* Use of a noninfectious surrogate to predict minute virus of mice removal during nanofiltration. *Biotechnology Progress* 34, 1213–1220 (2018).
415. Matsushita, T., Shirasaki, N., Tatsuki, Y. & Matsui, Y. Investigating norovirus removal by microfiltration, ultrafiltration, and precoagulation–microfiltration processes using recombinant norovirus virus-like particles and real-time immuno-PCR. *Water Research* 47, 5819–5827 (2013).
416. Shirasaki, N., Matsushita, T., Matsui, Y., Oshiba, A. & Ohno, K. Estimation of norovirus removal performance in a coagulation–rapid sand filtration process by using recombinant norovirus VLPs. *Water Research* 44, 1307–1316 (2010).
417. Pillay, S., Meyers, A., Williamson, A.-L. & Rybicki, E. P. Optimization of chimeric HIV-1 virus-like particle production in a baculovirus-insect cell expression system. *Biotechnology Progress* 25, 1153–1160 (2009).
418. Cervera, L. *et al.* Production of HIV-1-based virus-like particles for vaccination: achievements and limits. *Appl Microbiol Biotechnol* 103, 7367–7384 (2019).
419. Huang, X., Wang, X., Zhang, J., Xia, N. & Zhao, Q. Escherichia coli-derived virus-like particles in vaccine development. *npj Vaccines* 2, 1–9 (2017).
420. Lua, L. H. L. *et al.* Bioengineering virus-like particles as vaccines. *Biotechnology and Bioengineering* 111, 425–440 (2014).
421. Roldão, A., Mellado, M. C. M., Castilho, L. R., Carrondo, M. J. & Alves, P. M. Virus-like particles in vaccine development. *Expert Review of Vaccines* 9, 1149–1176 (2010).
422. Boonham, N. *et al.* Methods in virus diagnostics: from ELISA to next generation sequencing. *Virus Res.* 186, 20–31 (2014).
423. Wong, Y. -P., Othman, S., Lau, Y. -L., Radu, S. & Chee, H. -Y. Loop-mediated isothermal amplification (LAMP): a versatile technique for detection of micro-organisms. *Journal of Applied Microbiology* 124, 626–643 (2018).

424. Zhang, X., Lowe, S. B. & Gooding, J. J. Brief review of monitoring methods for loop-mediated isothermal amplification (LAMP). *Biosensors and Bioelectronics* 61, 491–499 (2014).
425. Feldmann, H. *Yeast: Molecular and Cell Biology*. (Wiley-Blackwell, 2012).
426. Thonart, Ph., Custinne, M. & Paquot, M. Zeta potential of yeast cells: application in cell immobilization. *Enzyme and Microbial Technology* 4, 191–194 (1982).
427. Hsu, B.-M. & Huang, C. Influence of ionic strength and pH on hydrophobicity and zeta potential of Giardia and Cryptosporidium. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* 201, 201–206 (2002).
428. Li, L., Mendis, N., Trigui, H., Oliver, J. D. & Faucher, S. P. The importance of the viable but non-culturable state in human bacterial pathogens. *Front. Microbiol.* 5, (2014).
429. Gunter, H. *et al.* Advances in quantifying microbial contamination in potable water: Potential of fluorescence-based sensor technology. *WIREs Water* 10, e1622 (2023).
430. Reygadas, F., Gruber, J. S., Ray, I. & Nelson, K. L. Field efficacy evaluation and post-treatment contamination risk assessment of an ultraviolet disinfection and safe storage system. *Water Research* 85, 74–84 (2015).
431. Hunter, P. R., Zmirou-Navier, D. & Hartemann, P. Estimating the impact on health of poor reliability of drinking water interventions in developing countries. *Science of The Total Environment* 407, 2621–2624 (2009).
432. Hutton, G. Monitoring 'affordability' of water and sanitation services after 2015: review of global indicator options. (2012).
433. UN. *Human Development Report 2006. Human Development Reports* https://hdr.undp.org/content/human-development-report-2006 (2006).
434. Ray, I. & Smith, K. R. Towards safe drinking water and clean cooking for all. *The Lancet Global Health* 9, e361–e365 (2021).
435. Blackden, C. M. & Wodon, Q. *Gender, Time Use, and Poverty in Sub-Saharan Africa*. (World Bank Publications, 2006).
436. Gender equality and sustainable development. (2014).
437. Chaúque, B. J. M. *et al.* Why do Low-Cost Point-Of-Use Water Treatment Technologies Succeed or Fail in Combating Waterborne Diseases in The Field? A Systematic Review. *Journal of Environmental Chemical Engineering* 110575 (2023) doi:10.1016/j.jece.2023.110575.
438. Bryan, F. L. Hazard Analysis Critical Control Point Approach: Epidemiologie Rationale and Application to Foodservice Operations. *Journal of Environmental Health* 44, 7–14 (1981).
439. Bryan F. L. & WHO. *Hazard analysis critical control point evaluations : a guide to identifying hazards and assessing risks associated with food preparation and storage.* https://apps.who.int/iris/handle/10665/37314 (1992).

440. Canadian Food Inspection Agency. Determining critical control points and their critical limits. https://inspection.canada.ca/preventive-controls/preventive-control-plans/critical-control-points/eng/1513353314619/1513353315119 (2021).

441. Shepard, Z. & Oyanedel-Craver, V. A review of the impact of testing conditions on the performance and quality control of locally manufactured, point-of-use ceramic water filters. *Environmental Science: Water Research & Technology* 8, 510–522 (2022).

442. WHO. Drinking-Water, Sanitation and Health. (2011).