master thesis – Water Science and Management

The risk of opportunistic pathogens in Dutch drinking water – A newly derived DALY approach for assessing the risk of *Legionella pneumophila*

Key concepts: drinking water quality, emerging pathogens, DALY, reverse QMRA

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Acronyms

Acronym	Meaning
C_{air}	The critical concentration of a pathogen expressed in CFU/L in the air
C_w	The critical concentration of a pathogen expressed in CFU/L in drinking water
CI	Confidence Interval
CFU/L	Colony forming unit per liter / organisms per liter
CSI	Clinical severity infection
D	Dose
DALY	Disability-adjusted life year
DB	Disease burden
DW	Disability weight
f_s	Susceptibility fraction
GBD	Global Burden of Disease study
ID	Infection dose
LD	Lethal dose
MC	Monte Carlo
PC_{baw}	Bacterial-air-water partitioning coefficient
P_{ill}	Probability of illness
$P_{ill\|inf}$	Probability of illness given infection
$P_{inf}(D)$	Estimated probability of an infection for a dose D
PPPA	Per person per annum
qPCR	Quantitative Polymerase chain reaction
QMRA	Quantitative microbial risk assessment
RIVM	Rijksinstituut voor Volksgezondheid en Milieu
SD	Standard deviation
YLD	Years lived with disability
YLL	Years of lost life
VBNC	viable but not culturable
WHO	World Health Organization

Abstract

The disease burden of waterborne pathogens is a growing concern within the Dutch water supply network. In 2011, Dutch health authorities have reported 476 infections with the opportunistic pathogen *Legionella pneumophila* causing Legionnaires' disease, causing death in 10% of all cases. The aim of this study was to express the risk of *L. pneumophila* infections with the DALY approach, combining mortality and morbidity in a single metric.

The critical concentrations for *L. pneumophila* in drinking water corresponding to the target of 10^{-6} DALY, were estimated with the reverse QMRA. For the reverse QMRA only one exposure scenario was described: the scenario of one person exposure to shower water inhaling bioaerosols with no background concentration of other aerosol producing devices within the shower room premise. The critical concentrations of fecal pathogens in drinking water as well as the critical concentration of *L. pneumophila* corresponding to an infection risk of 10^{-4} infections annually mandated by the Dutch Drinking Water Act, were estimated for the comparison to determine risk prioritization. In addition, the critical concentration of *L. pneumophila* in drinking water was estimated for three susceptibility fractions, constituting a different subset of the immunocompromised population.

Based on the 10^{-6} DALY approach, the results of the reverse QMRA show a mean critical concentration of *L. pneumophila* in drinking water of 23.6 CFU/L (95% mean confidence interval: 22.5 – 24.8 CFU/L), compared to 1.3 x 10^{-4} CFU/L and 1.05 x 10^{-4} CFU/L for *Cryptosporidium* and *Campylobacter* respectively and based on the DALY approach. The mean critical concentration estimated for the 10^{-4} infection risk approach yielded 1963.6 CFU/L, a concentration that is six log units less conservative compared to the 10^{-6} DALY as starting point. The correlation analysis of the parameters shower time, inhalation rate and air-to-water partition coefficient showed that the air-to-water partition coefficient has the largest influence in the reverse QMRA model and the highest uncertainty of these three variables, followed by the inhalation rate and the shower time, with the shower time deemed neglectable.

From the study it can be concluded that a large difference in critical concentration of *L. pneumophila* was observed between the DALY and the infection risk target. Drawing the critical concentration from the DALY yields a more stringent result compared to the model with the infection risk approach, indicating the significance of the disease burden as component in risk characterization of waterborne pathogenesis. While the critical concentration for *L. pneumophila* is in line with the *Dutch Drinking Water Act* for the infection risk (10^{-4} pppa), this is not the case for the 10^{-6} DALY, where a lower standard than the mandated 100 CFU/L is deemed more robust.

Because Legionnaires' disease results in a high number of fatalities, it is important to include the disease burden in risk assessments, which means that he reverse QMRA together with the 10^{-6} DALY is recommended for further research as instrument in risk assessment. For this purpose, more research is recommended towards the variability of the air-to-water partitioning coefficient, the risk of illness given infection and the disease burden quantification in terms of '*what is the state of compromised health*', in order to form ethically sound risk interventions.

Table of Contents

List of Figures

List of Tables

1. Risk identification of opportunistic pathogens

Human health relies partly on the environmental state, of which microbial growth in the drinking water distribution network continuously exposes humans to the risk of pathogenic adversities. Whereas risk managers have to a large extend addressed the exposure to fecal pathogens in the western hemisphere on the account of advanced treatment techniques, the exposure to opportunistic pathogens and their resistance to treatment processes has caused a rising number of clinical cases (Xing et al., 2021). Even though opportunistic pathogens are a relatively new matter of public concern – their attention has recently reached global scale – risk managers consider the risk quantification as too uncertain in their methodology to translate it into a public health concern in need of intervention (Xing et al., 2021; Bentham & Whiley, 2018).

Opportunistic pathogens, in contrast to pathogens with highly virulent strains, predominantly cause disease in immunocompromised individuals, but which, nonetheless are the root of serious infections and in a growing number of cases, result in death (van der Wielen et al., 2013). Opportunistic pathogens encompass different microorganisms such as *Pseudomonas aeruginosa, Mycobacterium avium or kansaii, Naegleria fowleri*, *Acanthamoeba* ssp., *Aspergillus fumigatus*, *Stenotrophomonas maltophilia* or *Legionella pneumophila*, where the latter, is the causing agent of the notorious Legionnaires' disease (Rasheduzzaman et al., 2019 and Hootsmans 2020). Not less of concern are infections with *P. aeruginosa* through exposure to contaminated drinking water, of which 17% are nosocomial, causing diseases such urinary tract infection, dermal infections, community-acquired pneumonia and occasionally the most extreme outcome, death (Rasheduzzaman et al., 2019).

1.1. Cases observed in the Netherlands

The *National Legionella Outbreak Detection Program* in the Netherlands emerged as a response to the outbreak of Legionnaires' disease caused by a hot tub at a flower show in Bovenkarspel in 1999, after which the national government declared infections with *Legionella* ssp. imperative to the public health agenda and requiring more scientific research. Since then, the program has reported 1,991 patients contracting the disease between 2002 and 2012. (Den Boer et al., 2015). In 2011, Dutch health authorities registered 476 cases of the disease, of which 334 cases were confirmed to be transmitted in the Netherlands, while the remaining

infections were traced back to overseas infections (reviewed in van der Wielen et al., 2013). The RIVM reported a total estimated number of 4407 infections between 2007 and 2011 (based on reported cases and assumptions on underestimation and underreporting) (Bijkerk et al., 2014). The largest share (69%) of the total legionellosis burden was observed in the age group of 45 to 69 years (Bijkerk et al., 2014). According to the RIVM (2020), the number of people contracted with Legionnaire's disease has since been on the rise, especially during the period 2013 to 2017 (Figure 1). For infections caused by other opportunistic pathogens, no verifiable data exists in terms of case numbers, mainly because diseases caused by these other opportunistic pathogens are not notifiable, resulting in disparity between the actual number and the number of unreported cases (RIVM, 2013).

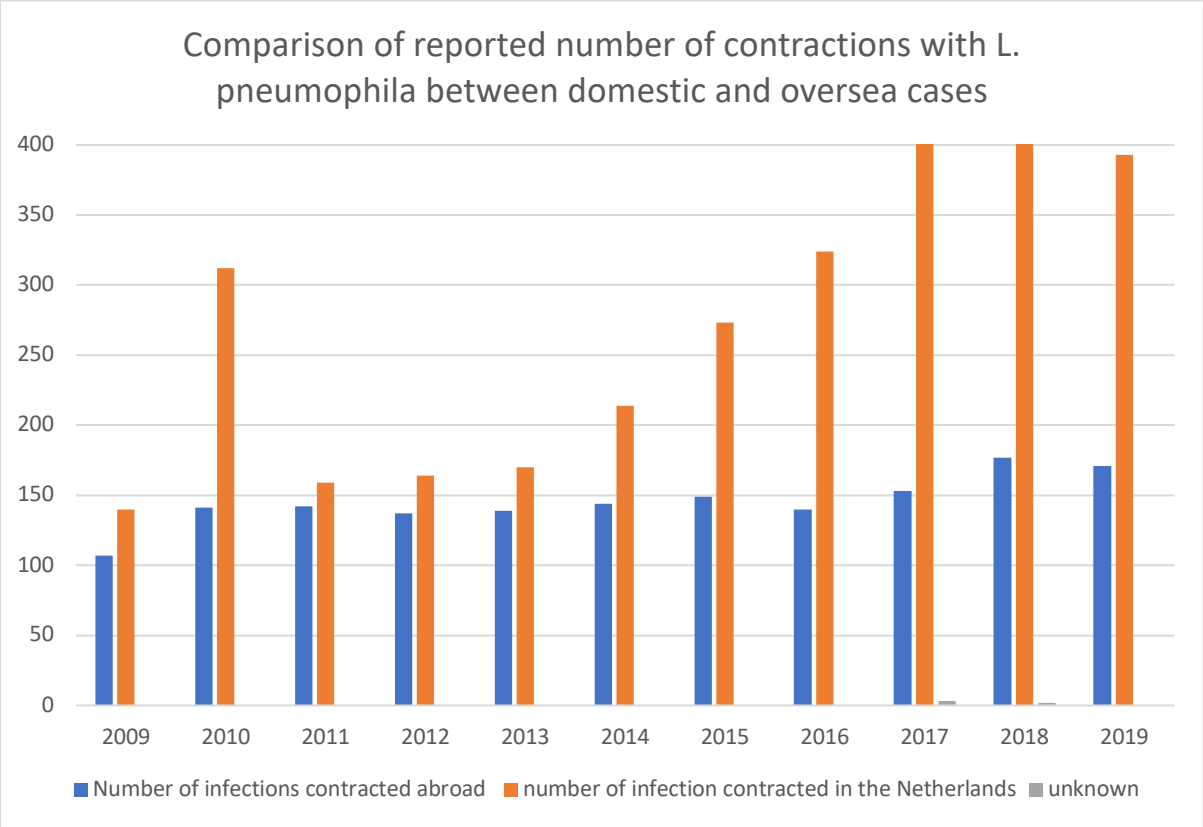

***Figure 2**. The number of infections with L. pneumophila observed in the Netherlands between 2009 and 2019 encompassing contraction in the Netherlands and abroad* (Data extracted from the RIVM, 2019)

As the link between opportunistic pathogens and Dutch drinking water has been investigated, with the result of *P. aeruginosa, L. pneumophila* and *A. fumigatus* showing matching phenotypical and genotypical strains, isolated from patients and observed in drinking water (Den Boer et al., 2015; van der Wielen & Wullings, 2019), their epidemiological significance has been affirmed as a result of these investigations.

1.2. Growth of opportunistic pathogens in drinking water systems

Unlike fecal pathogens, opportunistic pathogens can grow in the water distribution network, with growth mainly occurring in the biofilm on pipe surfaces (Schoen & Ashbolt, 2011). The main exposure route of opportunistic pathogens to humans is through inhalation of bioaerosols produced from taps and showerheads connected to the water supply network (Jingrang et al., 2016). The growth of opportunistic pathogens is a topic of public as much as scientific concern as their capability to multiply within biofilms gives them protection to disinfectants (Zhang et al., 2021). However, the growth of *L. pneumophila* is not only attributed to disinfectant resistance, because disinfectant residuals have been reduced to a minimum in the premises of the water distribution systems (Hamilton et al., 2018) or are absent in drinking water in some countries (e.g., the Netherlands, Denmark, parts of Switzerland and Germany).

While growth of opportunistic pathogens - except for *Legionella* - remains a poorly investigated topic (Jingrang et al., 2016), the interaction with other microorganisms, particularly intracellular replication in free-living amoebae (FLA) has been considered a potential mechanism and is even a perquisite for growth of *L. pneumophila* in drinking water systems (Hofbauer et al., 2018). As mentioned before, it has also been propounded that some opportunistic pathogens employ various survival mechanisms to thrive and multiply in biofilms. According to a study by Hsu et al. (2011), biofilms are nutrient rich systems compared to the rest of the water, thus providing growth conditions for free living amoeba and intracellular *Legionella*. The process of biofilm formation advances with the adhesion of microbial cells, forming a layer of microorganisms in an extracellular matrix of polymeric substances, that includes certain proteins (Drago & Toscano, 2017). Further research disaggregated bacteria into free-living and particle-associated bacteria, where the latter grow on the suspended particles in water and graze on the biofilm (Wang et al., 2018; Seiler et al., 2017). Unlike free-living bacteria, particle-based bacteria have been suggested to be larger and show greater resistance to disinfection by chlorine, ozone and ultraviolet. Hence, insufficient removal of suspended particles during the treatment process can be deduced as a growth factor, although more evidence is needed to confirm treatment insufficiencies (Wang et al., 2018).

The further need to investigate the risks of opportunistic pathogens is driven by rising temperatures – a growth factor – which has been linked to climate change. Whereas the causations of climate change have been widely explored in the context of our society

(Stockhause et al., 2019), little attention has been attributed to its impact on microbial growth in drinking water, whose temperature is subject to the dynamics of global warming (van der Wielen et al., 2013). The global mean surface temperature is predicted to continuously rise further through the 21st century. Different emission scenarios have been designed, predicting a rise in temperature varying from 1.1 to 6.4 °C depending on the distinct scenario applied (Meehl et al., 2007). Hence, this increase synchronously induces an increased growth of pathogens, as drinking water temperatures approach 25 to 30°C, the growth temperature of pathogens (van der Wielen et al., 2013). This putative mechanism has been confirmed by studies on the periodic changes of the *L. pneumophila* number, – with a larger count of the pathogen during summer – that link climate change to the growing risk of *L. pneumophila* (van der Wielen et al., 2013).

The second stressor emphasizes on the synergetic effect of population aging, and confoundingly, the improved health care, leading to an increased risk of opportunistic diseases: controversially enough, whereas improved health care increases life expectancy, the immune system of elderly is less active and weakens their chance to respond to an opportunistic infection. The same feedback is observed among people with serious, often immunosuppressing conditions, whose vulnerability to opportunistic pathogens increases, too (van der Wielen et al., 2013).

2. Concepts of risk assessment in epidemiological statistics

Poor drinking water quality is the cause of adverse health effects of varying degree and form. Attempts to design appropriate measures eliminating health risks are under constant discourse, examining the conceptualization of a risk assessment used as a foundation for feasible decision making on health targets (Havelaar and Melse, 2003). With the overall purpose to ascertain and as to achieve a reduction of health risks, the nature of a risk assessment must be understood as a tool of communication, improving the capability of decision makers to establish public health policies, including the quality of drinking water (Committee on improving risk analysis, EPA, 2009). Being cognizant of this fact, transparency and inclusion on all assumptions throughout the entire procedure is essential to attain effective risk interventions (Bosch, 2007).

Health

The concept of health has been subject to continuous revision over decades, reflecting medical status, economic welfare and cultural beliefs of the time and region. Defining health, nonetheless, is key to quantify health loss and to disaggregate different conditions impairing the quality of life (Havelaar and Melse, 2003). The practicality of modelling the loss of health has been attempted first by the *Global Burden of Disease Study by* Murray, and shortly after, has been under revision for the Netherlands by Stouthard et al (1997). Both models defined disability weights (DW) based on the ability to perform everyday-life-activities with procreation, pain, mobility, self-care and anxiety being the baseline for their assessment (Stouthard et al., 1997).

Tolerable risk

As the government being the mandating body in the Netherlands to design health policies, the responsibility falls onto them to reduce health risks to a tolerable degree. Establishing a tolerable degree of risk strongly correlates to sociometry and psychometry of the willingness to accept a risk, which is then translated into guidelines (Marszal, 2001). In terms of epidemiology of waterborne infections, an acceptable risk refers to the allowed exposure and concentration of a pathogen cognizant to be morbific (Havelaar and Melse, 2003).

Health targets

A health target guides the progress towards a policy goal with the objective of protecting public health. Health targets are designed with the intention to prevent the transmission of disease, including waterborne diseases. Because health targets often differ in their precision of scientific evidence, understanding the assumptions made for their development are crucial towards sensible decision making (WHO, 2011). A target in the domain of public health is grounded in the contemporary reality of a nation, where the redefinition of a target leads to continual cycle of evaluation and improvement through periodical risk assessments (Sphere Association, 2018).

Universal standard for a reference risk level

Setting one defined universal standard *of acceptable health risk* regardless of the risk characterization enables public health prioritization of waterborne diseases as it allows comparison between different agents and their health impact. Despite the complexity and range of factors determining *acceptable risk*, the need of a common metric– the *reference risk level* – is imperative for the provision of health impact assessment leading towards the achievement of meeting health targets (Havelaar and Melse, 2003).

For this, the disability adjusted life-years per person per year (DALY) as a reference risk metric has gained recognition in the field of waterborne disease transmission and will be further discussed in section 2.2 (WHO, 2021). When the burden of health is linked to an etiological agent in drinking water, exposure levels and maximum concentrations can be set according to the specific risk level (Havelaar and Melse, 2003). For the purpose of public health intervention, the quantification of the burden of a specific disease that is linked to drinking water quality is crucial to allocate monetary resources effectively (WHO, 2011).

2.1. Quantitative microbial risk assessment and waterborne pathogens

For further understanding of risk assessment for the pathogenesis of water-related exposure, the framework of the *quantitative microbial risk assessment* (QMRA) has evolved as a paradigm from chemical risk assessment and is largely equivalent to that in its procedure (van Leeuwen and Vermeire, 2007). In risk assessment, QMRA is a tool that furnishes transparent decision-making based on sound scientific evidence (Medema and Ashbolt, 2007). Broadly described, the QMRA follows the structure of four components, recognized as primary

constituents to a risk: *Problem definition, exposure assessment, health effects assessment and risk characterization* (WHO, 2016).

Foremost, the *problem definition* describes the purpose of the assessment given the health outcomes due to the microbiological hazard and pre-describes the exposure pathway of that hazard being investigated. The *exposure assessment* follows up on that, where the dose of pathogens transferred from source-to-human is characterized with the source concentration, the pathogen reduction rate through removal measures as well as the frequency and extend of exposure (WHO, 2016). The *health effects assessment* quantifies the impact of the health effects where the appropriate dose-response curve is an imperative instrument to describe the number of organisms one is exposed to, causing the pathogen-specific illnesses and symptoms (WHO, 2016). These are quantified in the DALY metric as disease burden (WHO, 2021). The last step of the risk assessment – *the risk characterization* – puts the risk into perspective in terms of validity of that risk and the uncertainties identified in the procedure of the risk assessment and how changes of the variables in the risk assessment affect the quantified risk output (WHO, 2016).

Normally, a QMRA uses the source concentration of a pathogen as starting point, and the result is a risk output (e.g. infection risk or DALY). A reverse QMRA can be performed as well, in which the starting point is the risk target which can be given in DALYs or as infection risk and the outcome is an allowable critical concentration in drinking water related to that risk target (Rasheduzzaman, 2019).

2.2. The fundament of the DALY

Within the field of QMRA for the application of water quality safety measurements, the infection risk is the putative method to identify the probability of disease occurrence. Different from that is the DALY approach, which quantifies the disease burden through the integration of the diverse health states observed and their magnitude with respect to age and pre-existing conditions leading to morbidity and mortality (Havelaar and Melse, 2003).

The DALY is an indicator combining the accumulative number of health years lost due to morbidity and premature death, where time is the unit metric (Corvalan et al., 2003). As for providing a clearer understanding, one DALY indicates the loss of one year at full health (WHO, 2021). Other than most health indicators such as life expectancy, the DALY attributes

the actual adverse health outcomes to the environment rather than the risk alone, for which drinking water is the immediate vehicle (WHO, 2017). Characterization of the DALY allows for a relative comparison among different health states and can fuel the prioritization of health interventions in policy making (Corvalan et al., 2003).

The DALY can mathematically be described with Equation 1, computing the difference between an ideal situation living in full health up to the standard life expectancy and the duration of living with the disease-incurred specific disabilities:

$$DALY = YLD + YLL \quad (1)$$

where YLD describes the *years lived with disability* and YLL the *years of lost life* due to premature mortality (Rasheduzzaman, 2019).

Years lived with disability (YLD)

The YLD component estimates the aggregated time lived with a disability, where the incidence of a disability is multiplied with the average duration of an adverse health impact. The latter can range from acute to irreversible damage (Corvalan et al., 2003).

The YLD describes the quantity of life which is multiplied with the quality of life expressed through disability weights according to Equation 2:

$$YLD = I \times L \times DW \quad (2)$$

where I is the number of incidences in a population or the number of cases of a disease during a specific period, L the average duration of a disability given in years and DW the disability weight (DW) (Corvalan et al., 2003). The DW is rated on a scale from 0 to 1 to characterize the severity of a disability, where 0 assigns a state of assumed full health and 1 indicates death. The *Global Burden of Disease* Study of 2013 currently encompasses a set of 235 disability weights describing the disease burden and which are periodically updated and added to (Feigin et al., 2013). Disability weights quantify the preferences in relation to the ideal state of health. Ideally to say, society judges suffering from some symptoms as "sicker" than others. For example, suffering from moderate diarrhea (DB = 0.188) is considered less severe than suffering from a migraine (DB =0.441). This simplification is sensible of the fact, that suffering

from severe dementia (DB = 0.449) is not regarded as a state of being half dead (Salomon et al., 2013).

Years of lost life (YLL)

The years of lost life are estimated by multiplying the number of deaths due to a specified health condition with the loss of life, as shown in Equation 3:

$$YLL = N \times L \tag{3}$$

where N indicates the number of deaths due to disease and L the standard life expectancy at the age of premature death given in years (Corvalan et al., 2003). Due to reasons of equality and the economic wealth gap between countries generally causing dissimilar life expectancies, the same life expectancy is applicable globally (Havelaar and Melse, 2003). The reference maximum life span is 80 and 82.5 years for men and women respectively (Corvalan et al., 2003). The rational for this selection is that this life expectancy defines the possible biological life span and which, in theory, every person can reach (Havelaar and Melse, 2003).

Besides the rational of choosing the appropriate life expectancy, age has been a point of frequent discussion, leading to the integration of age discounting in the DALY calculation. Based on the GBD study, younger age is attributed a higher value in terms of life quality and economic importance as reflected by society itself (Corvalan et al., 2003). Allocating value to age implies the parting from early to later years in life with its respective deterioration of the personal and societal value, where the assumption that everyone passes through all ages. By no means this should be understood as a legitimization to delay health intervention to a later state (Havelaar and Melse, 2003). As far as the DALY is concerned, a 3 to 5% age discounting rate is applied to account for the years of lost life in the future (Corvalan et al., 2003).

Infection risk approach

The annual risk of infection is determined by estimating the average number of the cumulative independent exposure events per individual per annum and the daily probability of developing a clinical illness for that exposure event (Benke & Hamilton, 2007). The infection risk approach and the DALY are the same with reference to the daily probability of an infection ($P_{inf.d}$), as both require the dose-response relationship for the pathogen under investigation (WHO, 2016). Equation 4 yields the average risk function for an individual:

$$P_{ann} = 1 - \prod_{i=n}^{n}(1 - P_{inf.i}) \quad (4)$$

where n describes the number of exposure events per annum and i denotes the individual infection risk per person per exposure event (Benke & Hamilton, 2007).

2.3. Infection risk and DALY – why choose for the DALY target

As described above, despite the complexity in the decision-making process on health targets a reference baseline is needed. The WHO advocates a DALY of 10^{-6} as the tolerable burden of disease target where the overall burden of disease is high due to multiple routes of exposure, varying health outcomes or due to high morbidity. This limit is equivalent to *one 'excess case of cancer per 100,000 people ingesting drinking water daily over a 70-year period'* (WHO, 2017).

According to Rasheduzzaman's and Hamilton's (2019; 2019) research where they used a reverse QMRA to compare the DALY and the infection risk approach for *P. aeruginosa and N. fowleri,* the severity of the disease burden of opportunistic pathogens becomes more apparent when using the DALY instead of the infection risk as the starting point. For Legionnaires' disease the high fatality rate (YLL) is the major contributor to the disease burden (Bentham & Whiley, 2018). In the Netherlands, the disease burden of Legionnaires' disease has been defined with a DALY of 4283 for the Dutch population and a DALY of 0.97 per person per annum (pppa) (Table 1) (Bijkerk et al., 2014)

Table 1. Comparison of the various water related illness with the ratio of DALYs to infections as metric, demonstrating the severity of Legionnaires' diseases compared to the other (diarrhea) causing illnesses (National Academics of Science, 2020).

Illness	**DALY per person per annum (pppa)**
Cryptosporidiosis	0.0015
Norovirus	0.003
Salmonellosis	0.003
Legionnaires' disease	0.97

3. Research aims and questions

As part of the joint research program of the Dutch drinking water companies and one Flemish drinking water company (BTO; *Bedrijfstakonderzoek voor waterbedrijven*), KWR conducts a risk assessment project, investigating the risk of opportunistic pathogens in Dutch drinking water in comparison to fecal pathogens. This is to see if health interventions are focused

Based on antecedent research, it has been determined that the presence of *L. pneumophila, P. aeruginosa* and *A. fumigatus* in drinking water pose a risk to public health in the Netherlands. With that point of reference, the Dutch drinking water companies investigate if additional research on the control of opportunistic pathogens in drinking water systems is required or if the risk of opportunistic pathogens is trivial compared to the risk of fecal pathogens and compared to sources other than drinking water (van der Wielen & Wullings, 2019).

In accordance with Article 21 of the Dutch Drinking Water Act (2009), '*tap water provided by the owner to consumers and other customers should not contain micro-organisms, parasites or substances to such numbers per volume or concentrations that these may comprise detrimental public health effects'*, opportunistic pathogens are included in the legislation, but guidelines on how to perform Quantitative Microbial Risk Assessment (QMRA) for opportunistic pathogens are absent, while being defined for surface water and for fecal pathogens (Schijven et al., 2011). This impedes the actual health impact assessment for opportunistic pathogens, questioning if their impacts are underestimated. This need to quantify the risk of opportunistic pathogens in a different manner and to assess whether current guidelines prioritize the pathogens causing the highest disease burden is hence imperative.

The issue is exacerbated by the limited data availability on opportunistic pathogens other than *L. pneumophila* as much as the heterogeneity of the disease states and the disparity in exposure route entails a significant drawback for the reverse QMRA to be conducted accurately (Bentham & Whiley, 2018). Because the Netherlands has a long history of studying *L. pneumophila* in drinking water and because research on this bacterium has produced attested information, this research is expected to yield well-founded results. The results are used to determine whether the QMRA with either the DALY or the infection risk approach can be conducted for other, more complex opportunistic pathogens and where the uncertainties of such

an approach lie. The risk assessment will be extended for research on *P. aeruginosa*, for which less attested data is foreseen.

Similar to the outcome of Rasheduzzaman's research, the critical concentration for Dutch drinking water is expected to be equally more conservative with the 10^{-6} DALY risk target instead of the 10^{-4} infection risk as starting point. Such a more conservative outcome has been reported for *L. pneumophila, P. aeruginosa and N. fowleri* (Hamilton et al., 2019; Rasheduzzaman's et al., (2019). To affirm this hypothesis, a reverse QMRA can compute the difference in critical concentration between the infection risk and the DALY approach using the above-mentioned risk targets.

Towards achieving this goal, the subsequent research question and five sub-questions are deemed pertinent towards determining the risk of opportunistic pathogens in Dutch drinking water:

Research Question

Can the risk of the pathogen *L. pneumophila* for Dutch drinking water be assessed using either the DALY and the infection risk and how relevant is this risk compared to other infections?

Sub-questions

1. What is the critical concentration limit for *L. pneumophila* in drinking water based on the 10^{-6} DALY per person per year WHO recommended target and how does this concentration relate to the annual infection risk target (10^{-4}) mandated in the *Dutch drinking water act*?
2. How does the risk of *L. pneumophila* compare to the risk of infections from the fecal index pathogens *Campylobacter*, *Giardia* and *Cryptosporidium* with the pre-defined target value of 10^{-4} per person per annum and based on the QMRA?
3. How does the DALY for *L. pneumophila* behave when including the immunocompromised fraction as a vulnerable subgroup of the Dutch population only?
4. What is the uncertainty of the parameters selected to perform the QMRA calculations and how do these uncertainties affect the result for both the DALY and infection risk approach?
5. What information and assumptions are needed to do make the reverse QMRA model more reliable?

4. Applying the reverse QMRA method for the risk characterization of *L. pneumophila* in drinking water

Inconsistent with conventional risk assessments, where measured pathogenic concentrations in drinking water are used to estimate the risk from exposure to microorganisms, this study's method was the 'reverse QMRA', where the critical *L. pneumophila* concentration is calculated using a bottom-up procedure and with the acceptable risk target as starting point (Rasheduzzaman et al., 2019). This is to determine the tolerable concentration in drinking water that conforms with a predetermined health target of *L. pneumophila.*

For two different targets – the DALY target of 10^{-6} pppa and infection risk target of 10^{-4} pppa – the risk of infection ($P_{inf.d}$) per event was computed. The parameters relevant for the model (Figure 2) were integrated into the reverse QMRA method to back-calculate the dose, from where, as the final step, the critical concentration *for L. pneumophila* in drinking water was calculated with a stochastic simulation in *R*. The input parameters (Table 3) have been outlined in various scientific studies. The remaining supplementary calculations required to back-calculate the critical concentrations follow the steps in Annex I – IV.

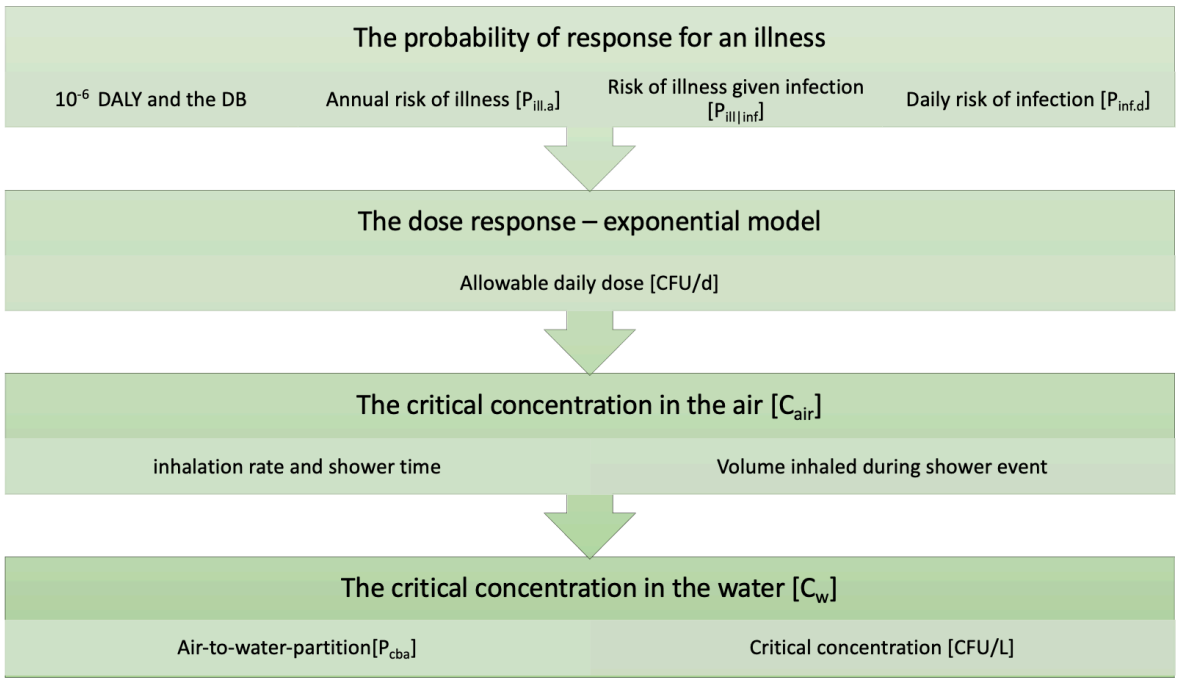

Figure 3. *Conceptualization of the reverse QMRA model with the Daly target and the disease burden as starting point*

4.1. The risk of annual illness

The risk of annual illness ($P_{ill.a}$) was calculated for both the DALY metric with the recommended WHO target of one micro-DALY (10^{-6}) and for the annual risk target of 10^{-4} mandated by the Dutch government (Havelaar and Melse, 2003 and Schijven et al., 2011). $P_{ill.a}$ were calculated with Equation 5 and 6 respectively, for which the disease burden was derived from Equation 1 – 3 and reported by the RIVM (Bijkerk et al., 2014).

$$P_{ill.a} = \frac{DALY}{(DB*100)} \tag{5}$$

where $P_{ill.a}$ is the risk of annual illness and DB is the disease burden per individual per annum.

Equation 6 is to solve for $P_{inf.yr}$ and is drawn from the annual infection risk target (10^{-4}) and was described under Equation 4.

$$P_{inf.yr} = \frac{DALY}{P_{ill|inf}} \tag{6}$$

where $P_{inf.yr}$ is the annual risk of infection and $P_{ill|inf}$ the risk of illness given infection.

4.2. The risk of illness given infection and the daily risk of infection

($P_{ill|inf}$) was used to calculate the daily risk of infection, where $P_{ill|inf}$ was determined based on Havelaar's (2014) study on the *Impact of Acquired Immunity and Dose-Dependent Probability of Illness on Quantitative Microbial Risk Assessment* and the through the RIVM estimated incidence number of cases reported positive for *L. pneumophila* between 2007 and 2011 (Bijkerk et al., 2014) as shown in Equation 7. The results for $P_{ill|inf}$ can be found in Annex III.

$$\pi = \frac{I}{f_s x N \; x \; E \; x \; P_{inf}(D)} \tag{7}$$

where I is the average number of illnesses, N the population size, E the number of exposures per person per year, $P_{inf}(D)$ the estimated probability of an infection for the dose D and π the fixed probability of illness given infection. $P_{inf}(D)$ was estimated as shown in Equation 8.

$$P_{inf}(D) = 1 - e^{-r \, x \, D} \tag{8}$$

where *r* is the dose-fitting parameter and where *D* equals one according to Havelaar's (2014) presumption that one cell is always present and can cause infection resulting in immunity. Immunity is assumed to be effective in an individual for a year (Havelaar et al., 2021). $P_{ill|inf}$ was estimated for substituting the population fraction f_s that was calculated for its respective immunocompromised group. The assumption is, that the number of infections with *L. pneumophila* occur within the respective population fraction. This means that *I* is constant. The approach assumes that the immunocompromised population has a lower immunity, producing a higher value for $P_{ill|inf}$.

The daily risk of infection ($P_{inf.d}$) follows the WHO approach in reverse and was calculated using Equation 9.

$$P_{inf.d} = \frac{P_{ill.a}}{(P_{ill|inf} * 365)} \quad (9)$$

4.3. Dose-response

The dose-response assessment reproduces the relationship between $P_{inf.d}$ and a dose (d), that is to say, the probability for causing adverse health impacts due to the exposure dose of a pathogen. For *L. pneumophila* the exponential model has been deemed as the most pertinent fit build on infection data from guinea pigs (Kusumawardhana et al., 2021). The dose-response model is derived from the pathogenesis principle of the single-hit infection theory, meaning that a single cell can cause an infection (Weir et al., 2020). The underlying rational is that each organism is subject to the same and constant survival probability, given by the dose-response fitting parameter *k* (given in $cells^{-1}$) (Kusumawardhana et al., 2021). The dose-response fitting parameter *(k)* for *L. pneumophila* is listed in Table 3. and was derived from a study by Armstrong and de Haas (2007).

The exponential model is given in Equation 10, where the daily risk of infection is derived from Equation 10, solving for the dose (*D*), and which can be interpreted as the allowable dose of organism a person can be exposed to per day. The supplementary information is given in Annex IV.

$$P_{inf.d} = 1 - e^{-k \, x \, D} \quad (10)$$

where $P_{inf.d}$ is the daily risk of infection, *k* is the dose-response fitting parameter and *D* the dose (Kusumawardhana et al., 2021).

4.4. The critical concentration

In the final step the critical concentration of *L. pneumophila* in the air through bioaerosols is calculated with Equation 11, solving for the parameter C_{air} [CFU/m^3].

$$C_{air} = \frac{D}{IR \; x \; T \; x \; 1000} \tag{11}$$

where *D* is the allowable dose inhaled (CFU), *IR* the inhalation rate (m^3/min), *T* the duration of the exposure (shower) event (min), and 1000 is in the conversion factor from m^3 to liter in order to estimate the total inhaled volume in liter. The assumed number of (shower) events per person per day is one. The inhalation rate and the duration of the exposure event are distributions and were simulated with Monte Carlo (MC) in *R Script* to find the best fitting mean and minimum critical concentration in drinking water (Annex VI).

The critical concentration in water (C_w) is solved by the division of C_{air} and the bacterial water-to-air partitioning coefficient (PC_{bwa}) as with Equation 12.

$$PC_{BWA} = \frac{C_{air}}{C_w} \tag{12}$$

The PC_{BWA} distribution was provided by Chattopadhyay et al., (2017), who created a controlled shower room to measure the concentration of the pathogen *P. aeruginosa* detected in air and water for the partition of bioaerosols. The bacterial characteristics of *P. aeruginosa* – rod-shaped and Gram-negative – have been deemed a sufficient match to estimate the fate of *L. pneumophila*. The fraction of respirable aerosols (FRA) for *P. aeruginosa* is 99.9% according to Chattopadhyay et al., (2017) was deemed neglectable.

The variability in the shower water temperature is obliquely accounted for in the variation of the bacterial water-to-air partitioning coefficient PC_{bwa} discerning between 10, 25 and 37 degrees Celsius (Figure 3) (Chattopadhyay et al., 2017).

The result was simulated with 10,000 iterations in Monte Carlo. Once C_{air} was calculated from the allowable dose, the critical concentration in water was simulated with 10,000 draws to address parameter uncertainty, from which, the critical concentration of *L. pneumophila* was

drawn. The MC simulation equips drinking water companies in the Netherlands with a range of possible outcomes. The critical concentration gives a reference maximum of the allowed concentration of *L. pneumophila* in the premise water supply that conforms with the DALY target of 10^{-6} and the infection risk target of 10^{-4}.

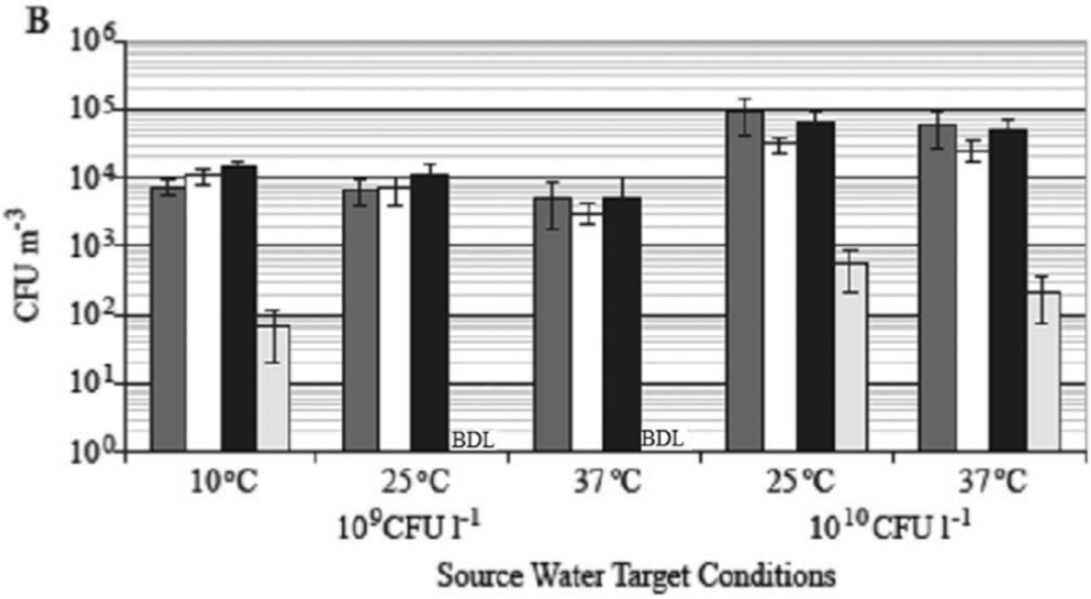

Figure 3. *Relation of the measured concentration of P. Aeruginosa in water and its measured concentration in bioaerosols in the air after partition. The coloring indicates the location of the sampler in shower room set-up. BDL indicates below detection limit of the sampler device (Chattopadhyay et al., 2017)*

The spearman rank correlation analysis was conducted, in order to assess the greatest influence of the model's variables; the inhalation rate, the average shower time and the partitioning coefficient on the dependent target variable, that is to say, the mean critical concentration in drinking water. The other parameters of the model were not included in the spearman rank correlation analysis and were given as fixed values.

The critical concentration for *L. pneumophila* will be compared to the critical concentration estimated for fecal pathogens following the same reverse QMRA method as to delineate how the risk of *L. pneumophila* relates to fecal pathogens.

An alternative health target was estimated to meet the infection risk target of 10^{-4} mandated in the Dutch Drinking Water Act (Schijven et al., 2011). Because the infection risk target of 10^{-4} is accepted by the Dutch government, a DALY derived from that target and the disease burden of *Campylobacter* was used to estimate the critical concentration for *L. pneumophila.* As a third target, the DALY of *Campylobacter* was estimated, where the risk of illness (P_{ill}) was replaced by the target of 10^{-4} (Annex V). The alternative health target was computed with Equation 13.

$$DALY_{Campylobacter} = P_{ill} \times \mathrm{DB} \times f_s \times 100 \quad (13)$$

4.5. Risk of the immunocompromised population for *L. pneumophila* from drinking water

Because *L. pneumophila* causes opportunistic infections, the disaggregation of population data is to address if a more conservative concentration limit for *L. pneumophila* is needed to protect the immunocompromised fraction of the Dutch population (referred to as the immunocompromised in the remainder of this thesis). Dose-response relationships cannot model the etiology of the heterogenous disease outcomes of the individual hosts effects because of their derivational limitations (Weir et al., 2020). Hence, the physiological effects have been estimated as susceptibility fractions (f_s) of the population and are summarized in Table 2. The corresponding in-depth calculations are given in Annex I.

The prevalence of daily smoking and the age are detrimental for the assessment on the immunocompromised fraction and are indicated in percentage of the total Dutch population. People fitting in the age group 50 years and older are considered immunocompromised due to a reported prevalence of 75 – 80% Legionnaires' disease cases observed at this stage of life. A similar risk is reported among male, accounting for 60 – 70% of all reported infections with *L. pneumophila* (WHO, 2018) forming a second risk group. Smoking has been linked to respiratory infections with the fraction of daily smokers (17.2% as of 2017) being 50 years and older of the Dutch population representing a third risk group (Trimbos Institute, 2017).

Table 2. Susceptible fractions of the Dutch population that were applied according to their risk factors and their adjusted risk of illness given infection (ANNEX I)

Susceptible fraction (f_s)	**Risk factors**
100%	none
7.1%	Age < 50; smoking
41.3%	Age < 50
19.7%	Age < 50; male

Table 3. Overview of exposure scenario input parameters and distributions derived from different scientific literature, applied in the simulation of the reverse QMRA model. The first block lists parameters needed for the probable risk of infection, the second block lists parameters for the dose-response of the exponential model. The third block lists the distributions, and the fourth block lists the parameters to estimate the critical concentration

Variable	Parameter	Value	Distribution	Unit	Reference
Disease burden	DW [DALY/case]	0.97	-	pppa	National Academics of Science (2020)
Risk target - DALY	-	10^{-6}	-	-	Havelaar and Melse, (2003)
Disability weight	-	0.09*	-	-	Stouthard et al. (1997)
Risk target - infection risk	-	10^{-4}	-	-	Schijven et al., (2011)
Number exposure events/annum	N_1	365	-	Days	-
Dose-response fitting parameter	K	0.059	-	-	Armstrong et al., (2007)
Dose	D	-	-	CFU	-
Partitioning coefficient	PC min – max	4.56×10^{-6} – 1.69×10^{-5}	Uniform	L/m^3	Chattopahyay et al. (2017)
Inhalation rate	IR min – max	min= 0.0042 max=0.017 mode=0.013	Triangular	$m^{3/}min$	Dean et al., (2020)
Duration exposure event	T	Mean=7.8 sd=0.4	Normal	min	Hamilton et al., (2019)
Number of events per day	N_2	1	-	-	-
Critical concentration	C_w	-	-	CFU/L	Kusumawardhana et al., (2021)
Monte Carlo	count	10,000	-	person	-

* The Disability weight was converted from the disability weights for diseases in the Netherlands to the GBD weights, which are weighted according to a different scale. The weight reported by Stouthard et al., (1997) corresponds to 0.91.

5. Exposure route and research scenario for *Legionella pneumophila*

5.1. Limitations of the research scenario

For the risk assessment of *L. pneumophila* in drinking water, only one exposure scenario was considered, scilicet the exposure due to shower events as simplified in Figure 4. In concordance with Hamilton's (2019) conclusion, shower events pose the highest risk for *Legionella* infections through drinking water. The accumulative influence of various exposure routes from other aerosol-producing water applications within the close vicinity of the shower head application (e.g., due to toilet flushing) is neglected along with the preceding assertion, that shower cells are spatially separated from toilets (Quang et al., 2021). Potential exposure routes from aerosol producing devices exterior of the domestic environment such as cooling towers, hot tubs, or spray irrigation are beyond the scope of this research. Still, these other sources could also contribute significantly to *Legionella* infections.

Legionnaires' disease was the only disease outcome considered for the scenario used in the reverse QMRA, meaning that Pontiac fever is excluded from this study for reasons of neglectable quantifiable health effects compared to Legionnaires' disease (Quang et al., 2021).

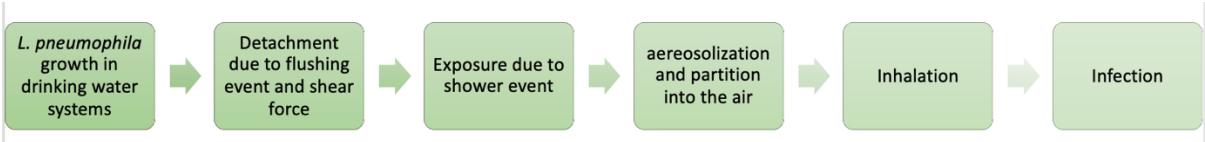

***Figure 4**. Conceptual model on the exposure assessment and exposure route for the reverse QRMA of L. pneumophila*

5.1.1. Growth of *L. pneumophila* in drinking water systems

In the Netherlands – a country characterized by moderate climate – *L. pneumophila* growth occurs solely in the premise plumbing system in buildings as drinking water temperatures in these premises plumbing systems reach the range for *L. pneumophila* growth (25 to 45°C) (Schoen et al., 2011 and Huang et al., 2020).

Irrespective of the variation in temperature, larger buildings like hotels, offices or hospitals show that *L. pneumophila* can thrive in premises plumbing systems in the Netherlands due to stagnant water (den Boer et al., 2014). This research does not distinguish risks between neither building types nor their variation in stagnation time due to the utility of the building (e.g., an office building is largely vacant during weekends).

Variation due to the heterogeneity of the water premise, where several factors such as the difference in pipe material, varying stagnation periods or water aging and water temperatures were not considered (although the water temperature is indirectly accounted for in the PC_{bwa}) (van der Kooij, 2013). The associated variation of the actual detected *L. pneumophila* concentration in the Netherlands was discussed in Chapter 7.3.

5.1.2. Assumptions made for the number of exposure events

The average number of exposure events per individual per year is prescribed with 365 events, assuming that each person in the Netherlands showers once a day, where the unit metric is expressed in days (Rasheduzzaman et al., 2019). No information was given on the presence of *Legionella*, leading to the assumption that *Legionella* is present at each shower event.

Variations of the average shower time are based on the data reported by Hamilton et al., (2019). The exposure to *L. pneumophila* in the shower room after the shower event lapsed until the point that all aerosols have been removed is considered neglectable compared to the risk of inhaling aerosols with *Legionella* during showering. Likewise, this study focuses on the exposure event of one person alone in the shower room.

5.1.3. Aerosolization, partition and inhalation

The number of *L. pneumophila cells* in water is reduced when partitioned from the bulk water into aerosols dispersed into the air, where only aerosolized cells in the range of 1–10 µm in diameter are pertinent for the inhalation process, as larger aerosols are beyond the respirable range and hence, impuissant to deposit in the alveolar region either via mouth or nose (Schoen and Ashbolt, 2011).

While the scenario includes the differences in human behavior relevant to this shower scenario, (shower time duration and inhalation rate), it does not differentiate between aerosolized particle deposition in the alveoli due to dissimilarities of oral and nasal breathing patterns (Chattopadhyay et al., 2017). Other factors such as variations in aerosol generation, the flow rate of hot water from the shower head, the distribution of aerosols of different size and the size of the average size of the shower room are accounted for in the partitioning coefficient (Chattopadhyay et al., 2017).

5.2. Health effect's assessment

5.2.1. Defining the number of cases observed in the Dutch population

With reference to the population at risk, the Dutch population as a whole and the number of immunocompromised people representing a vulnerable group have been identified (van der Kooij, 2013). For that purpose, this research is performed within the following framework:

The proportion of foreign people travelling to the Netherlands acquiring Legionnaires' disease in the Netherlands but detecting the infection after travelling to their country of residence is beyond the scope of this research due to the futility to track these infections. The Dutch health authorities registered 476 cases of the disease in 2019 and of which 142 cases – corresponding to 30% – were traced back to overseas infections (RIVM, 2020). It is assumed that the number of cases with *Legionella* infections contracted abroad were accounted for in the DALY estimation for *Legionella* by the RIVM through the multiplication factor chosen to adjust for underestimation of the number of Legionnaires' disease cases (Bijkerk et al., 2014).

For all risk groups, a 'steady-state' demographic framework is assumed, signifying a static population structure in terms of age and size remaining unimpaired to migration patterns, and diminishing natural immunity to potential new medical discoveries that improves health care in the future. Further, the steady reduction of smokers in the Netherlands remains unaddressed. Neither age discounting nor co-morbidity was applied in the Dutch infection study for the RIVM to calculate the disease burden of *Legionella* in the Netherlands (Bijkerk et al., 2014).

5.2.2. Understanding the disease burden

The DALY value for the disease burden of *Legionella* was estimated by the RIVM in the past and was based on the method applied by Havelaar and Melse (2003). Legionnaires' disease was reported on as the symptomatic infection in the *Appendix: State of Infectious Diseases in the Netherlands (2013), p.86* as depicted in Figure 5, and was attributed with a value of 0.97 DALY pppa.

The model by Stouthard et al., (1997) was created with the following input parameters: distribution of health states in health outcomes, risk to develop that health outcome, disability weight (DW) and disease duration. The values for these parameters were derived from various scientific literature. The DWs (and disease duration) were attributed to five health states: (1) Legionnaires' disease fatigue, (2) post-traumatic stress disorder, (3) concentration problems

and memory loss, (4) muscle joint pain and muscle weakness and (5) death (Figure 5; Bijkerk, 2014), and were derived from various scientific literature. The disability weight elicited for an infection with *L. pneumophila* is 0.09 and corresponds to a disease stage of a two-week pneumonia episode in an otherwise healthy year (Stouthard et al., 1997).

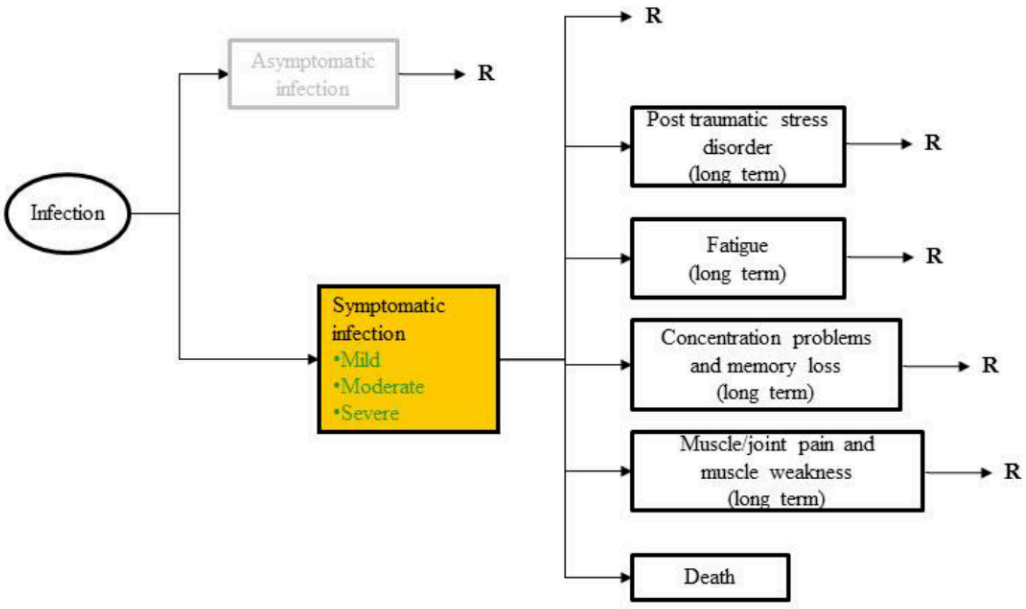

Figure 5. *Pathogen-based outcome tree of Legionnaires' disease (RIVM, 2013)*

6. Results of the risk characterization for *L. pneumophila*

6.1. The critical concentration for *L. pneumophila*

The distribution of the critical concentration for *L. pneumophila* estimated with the reverse QMRA and with a health target of 10^{-6} DALY pppa is given in the histogram in Figure 6, where each iteration can be interpreted as a hypothetical test subject showering within the framework of the given parameter distributions. The range in critical concentration is 7.9 – 105.1 CFU/L, with a concentration density occurring within the first quarter towards the minimum and digressing towards the maximum. The mean critical concentration for *L. pneumophila* is 23.6 CFU/L (95%CI mean = 22.4 – 24.8 CFU/L). In other words, a mean critical concentration of 23.6 CFU/L conforms with the health target of 10^{-6} DALY, assuming a disease burden of 0.97 DALY pppa estimated for Legionnaires' disease.

The lowest result of the MC simulation is 7.9 CFU/L and can be understood as a benchmark. The minimum is a statistical variability based on the distributions, meaning that bootstrapping would lead to a slightly different minimum, as the normal distribution is not within a range of limited minimum and maximum. A legislation subject to this criterium – the minimum critical concentration – would protect the whole population from an infection with *L. pneumophila*, assuming that the given population data can be extrapolated to the entire Dutch population.

The critical concentration is lower than the mandated concentration of 100 CFU/L *Legionella* ssp in the *Dutch Drinking Water Act,* causing a non-trivial discrepancy in drinking water quality standards, which further is considered ineffectual with respect to the 10^{-6} DALY health target (Schijven et al., 2011).

A profile was simulated for the relation between the critical *L. pneumophila* concentration in drinking water and the distribution of the inhalation rate, shower time and PC_{baw} (Figure 7), where every dot represents a simulated showering person with a random combination of the inhalation rate, shower time and partitioning coefficient for that person. The comparison of all three graphs shows the Pareto efficiency favoring a situation where no individual will encounter DALY loss under the preferred criterion of the minimum critical concentration estimated for *L. pneumophila* (7.9 CFU/L).

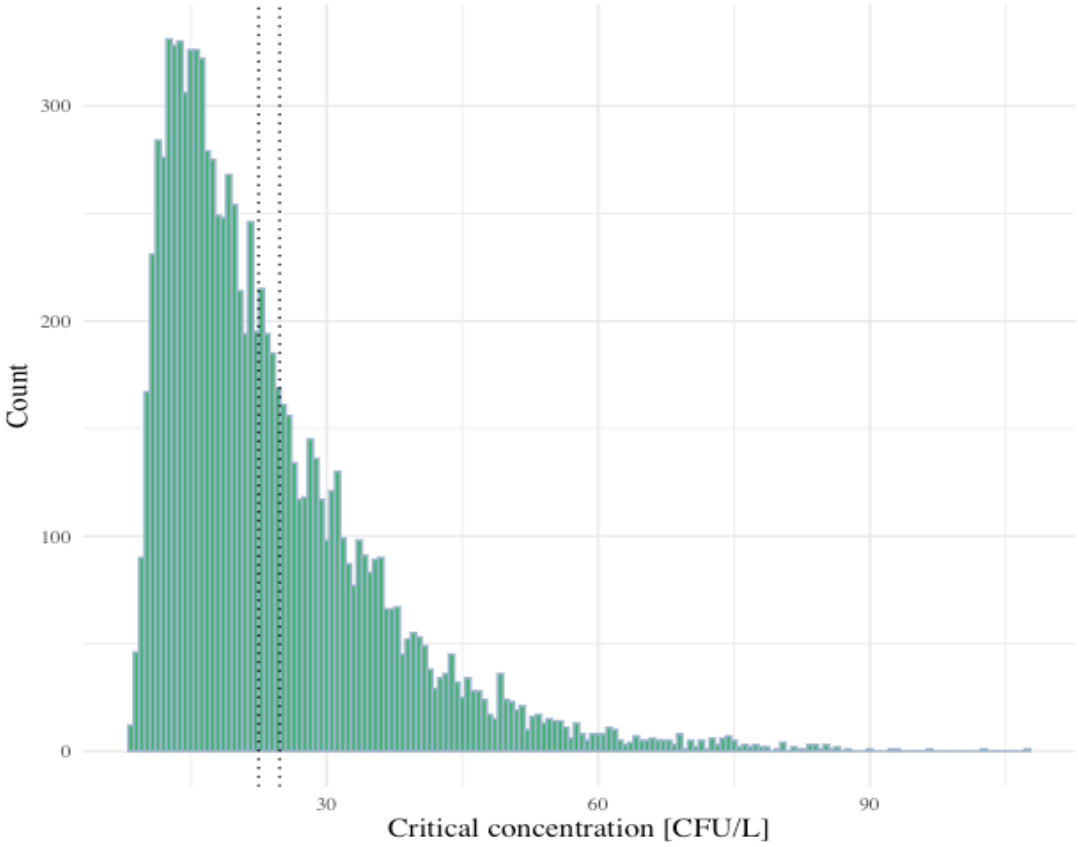

Figure 6. *The distribution of the critical concentration is illustrated on the x-axis in green for 10.000 iterations with the count shown on the y-axis. The dotted lines indicate the mean CI 95%*

The inhalation rate to critical concentration (Figure 7.1) shows a higher critical concentration for people with a lower inhalation rate, where, in addition, for a lower inhalation rate a larger variation in critical concentration is observed. A higher inhalation rate is mostly observed in children, people with an underlying disease, suffering for instance from respiratory diseases, and the elderly. This means that these persons (especially people with an underlying disease and the elderly, because children are less vulnerable for Legionnaires' disease) would benefit from a more conservative critical concentration for *L. pneumophila* in drinking water.

The distribution of PC_{bwa} was estimated for a for varying water temperatures (Figure 3) (Chattopadhyay et al., 2017). The partition of aerosols containing cells of *Legionella* from water into air is illustrated in Figure 7.2. The mean critical concentration for *L. pneumophila* in drinking water is higher for a lower PC_{bwa}, and lower for a higher PC_{bwa}. Equivalent to the observation of the inhalation rate against the critical concentration, the variation in critical

concentration is larger for a lower PC_{bwa}. Because the PC_{bwa} is in the denominator of Equation 12, a higher PC_{bwa} reduces the outcome of the equation through the mathematical division.

The distribution of the critical concentration of *L. pneumophila* in drinking water against the shower time is given in Figure 7.3. The critical concentration for *L. pneumophila* in drinking water peaks between 10 – 40 CFU/L for a median shower time of 7.8 minutes and decreases for both lower and higher shower times. This is solely incidental, as more draws of the MC simulation are centered around the median shower time. Compared to Figure 7.1 and 7.2, outliers (a deviation from the mean in the order of at least one log unit) are observed more frequently, increasing the variability in critical concertation per shower event, with a low uncertainty within the variability. The overall distribution is heterogenous from the Figure 7.1 and 7.2, as no discernable effect between a shorter and longer shower time on the critical concentration has been observed bases on the faint slope of the bottom pareto front, presumably owing to the narrow window of the shower time. For a significantly longer but unrealistic shower event (e.g., 7000 minutes), an effect in the order of approximately two log units on the *L. pneumophila* critical concentration was observed.

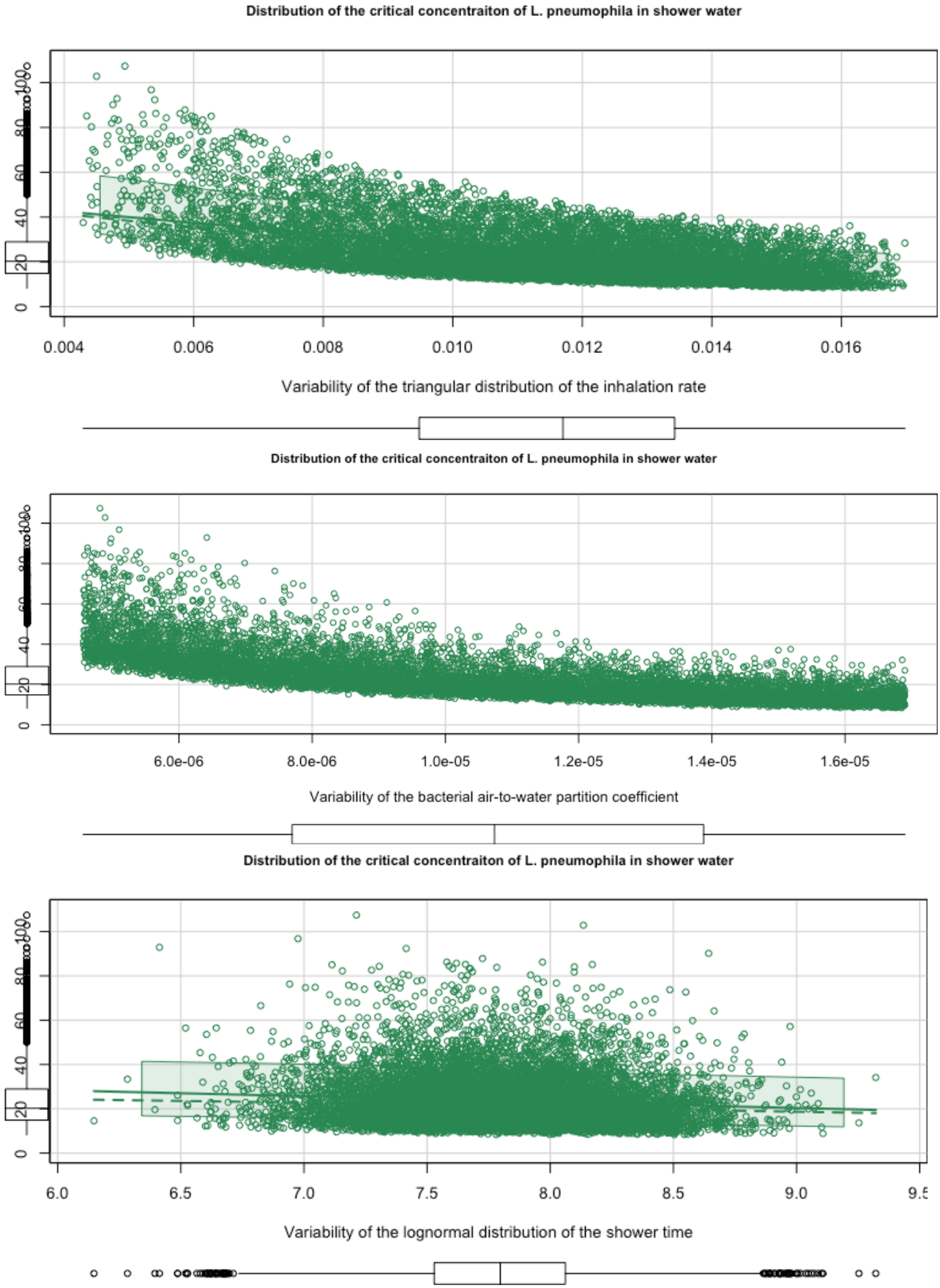

Figure 7. *Distribution of the critical concentration for L. pneumophila in respect to the model input parameter distribution.* ***Figure 7.1.*** *the critical concentration in respect to the triangular distribution of the inhalation rate;* ***Figure 7.2****. the critical concentration in respect to the partitioning coefficient;* ***Figure 7.3****. the critical concentration in respect to the shower time*

6.1.1. The Disease Burden with the DALY target of 10^{-6} and the infection risk target of 10^{-4} given their estimated population fractions

The critical concentrations for *L. pneumophila* in drinking water were estimated for different population fractions that typify the immunocompromised population and were compared to the critical concentration for the whole population fraction (f_s = 100%) in Figure 8. The susceptibility fractions are an estimate for the population fractions with the predefined characteristics of an immunocompromised person, and for which $P_{ill|inf}$ was modified. The first column shows the mean critical concentration for the whole population. The mean critical concentration for an immunocompromised male of age 50 (and older) is 4.6 CFU/L, whose characteristics apply for 19.7% of the Dutch population as shown in the second column. For a population fraction where age is the only valid factor for a person to be considered immunocompromised person, the susceptibility fraction increases to 41.3%, which results in a slightly higher critical concentration of 9.6 CFU/L (column 3) For a population fraction comprising people being at last 50 years old and smoking, the critical concentration is 1.6 CFU/L, which applies to 7.1% of the Dutch population (fourth column).

The difference in the *L. pneumophila* critical concentration between a random healthy person and an immunocompromised specimen being at least 50 years old and smoking is 22 CFU/L. In the latter case, the critical concentration estimated for a population fraction is spread over a smaller number in the population with that fraction showing a higher occurrence of infections with *L. pneumophila* compared to f_s = 100%. This indicates the interdependency with the potentially affected number of people. A priory, a person being immunocompromised benefits from a lower critical concentration and a QMRA that incorporation of the disease burden.

The critical concentration of *L. pneumophila* was also calculated for the 10^{-4} infection risk for different population fractions and is given in Figure 9. An analogous trend is observed: for a smaller population fraction the critical concentration estimated for *L. pneumophila* decreases in a comparable order of magnitude. While the mean critical concentration estimated with the infection risk approach for the average population is 1963.6 CFU/L, the mean critical concentration for smokers over 50 years old is 157.8 CFU/L.

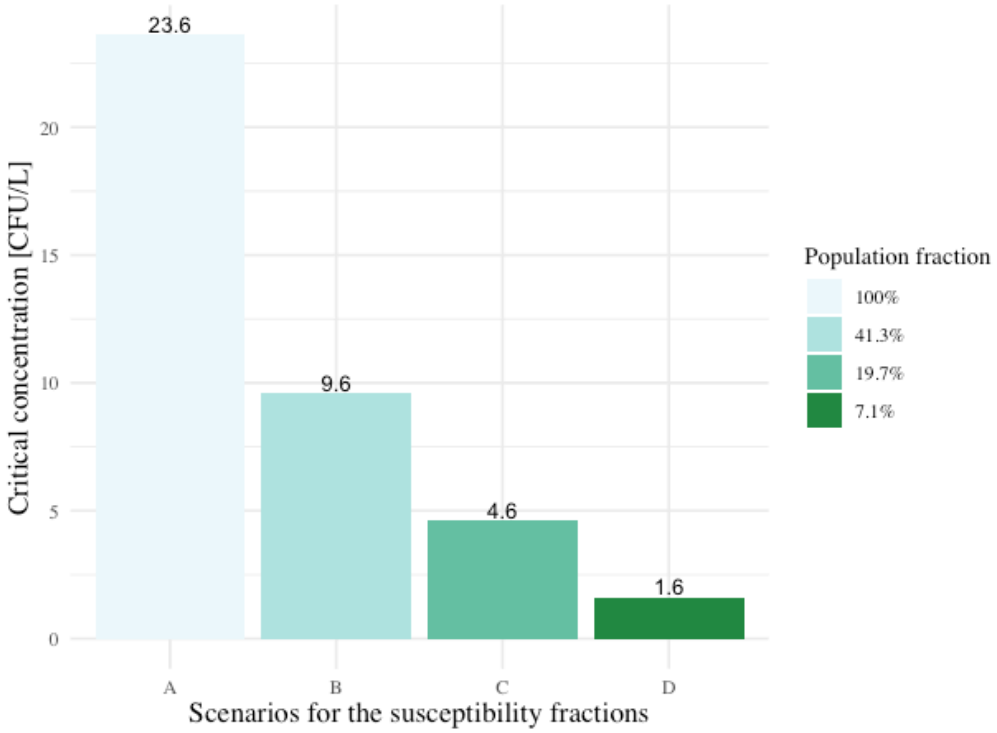

Figure 8. *Comparison between the critical concentration in respect to different susceptibility fractions estimated for L. pneumophila with the 10^{-6} DALY target (A=no immunity status; B=age<50; C=age<50, male; D=age<50, smoking)*

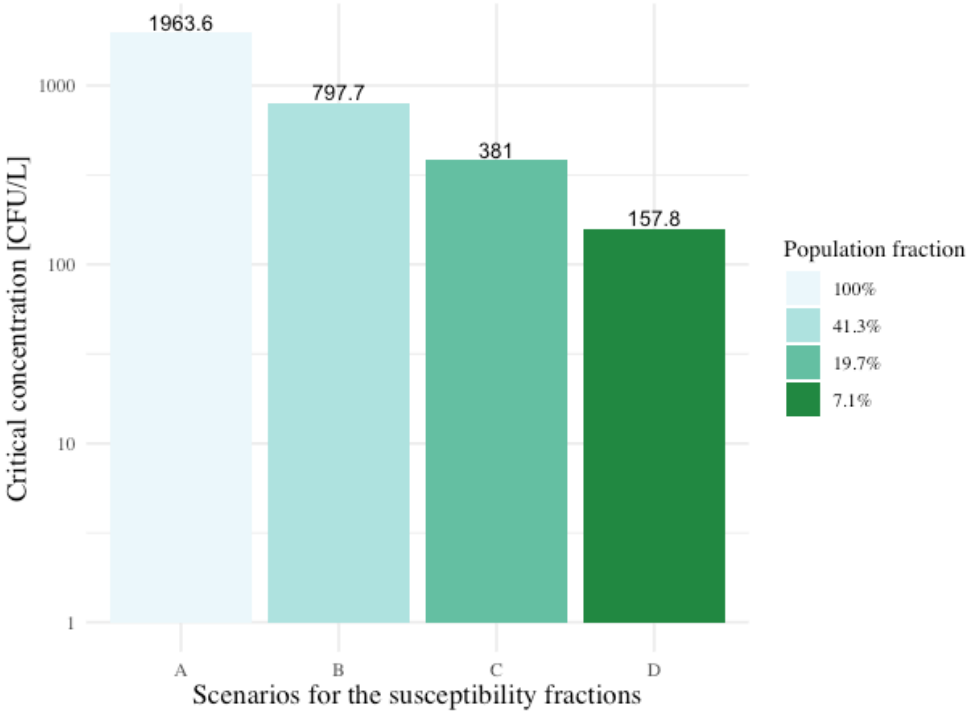

Figure 9. *Comparison between the critical concentration in respect to different susceptibility fractions estimated for L. pneumophila with the infection risk approach and the health target of 10^{-4} (A=no immunity status; B=age<50; C=age<50, male; D=age<50, smoking)*

6.1.2. The comparison between different health targets

In between the infection risk target (10^{-4}) and the DALY target (10^{-6}), a third target has been used to estimate the critical concentration of *L. pneumophila:* The target was adapted for the DALY of 4.64 x 10^{-7} of the *Campylobacter* bacterium, which was calculated from the mandatory 10^{-4} infection risk for *Campylobacter*. In Figure 10, the comparison of the three health targets is shown, where the health target derived from the DALY of *Campylobacter* shows a more conservative critical concentration compared to the critical concentration derived from the 10^{-6} DALY target. The critical concentration of 3.3 CFU/L is one- and three order of magnitude lower compared to the DALY target of 10^{-6} and the infection risk target of 10^{-4} respectively.

Because the DALY target was calculated from the disease burden of *Campylobacter* and the risk of illness derived from the infection risk target of 10^{-4} for the given risk of illness given infection ($P_{ill|inf}$), the critical concentration of 3.3 CFU/L also corresponds to the legislative health target of 10^{-4} mandated by the Dutch government and the health risk for *Campylobacter*.

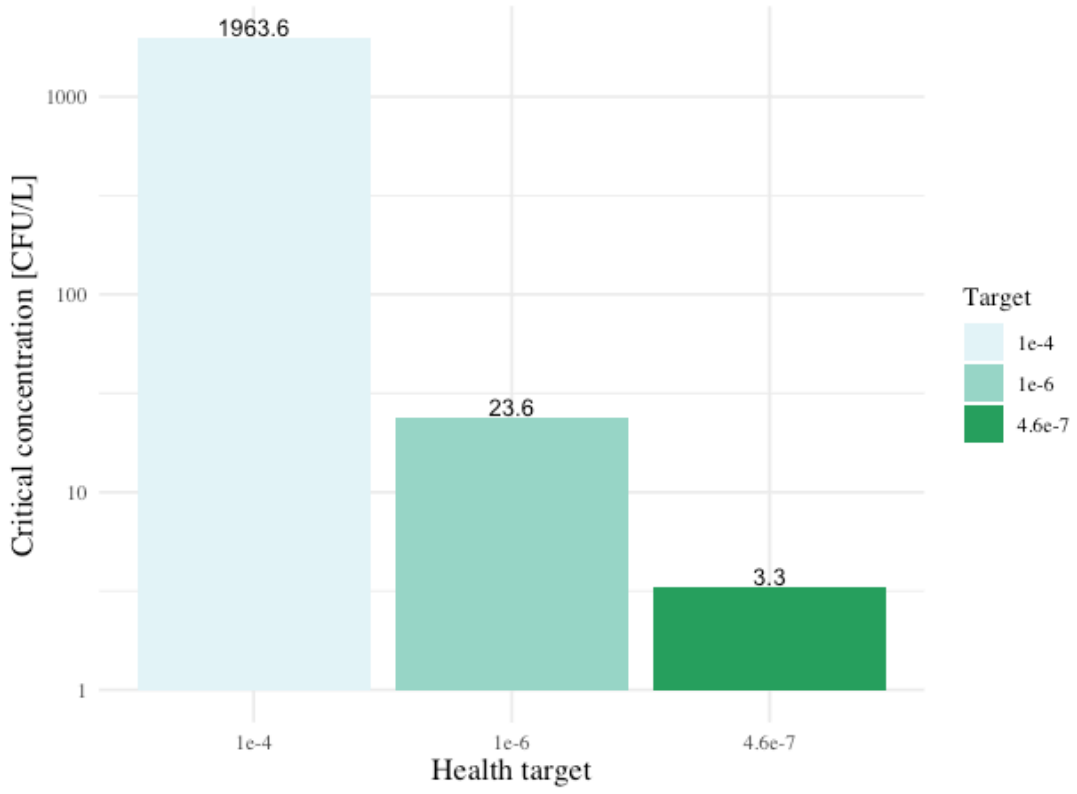

***Figure 10**. Comparison of the critical concentration estimated for different health targets. From left to right: the DALY target of 10^{-6}, the infection risk target of 10^{-4} and the target corresponding to the DALY of the infection risk and the disease burden of Campylobacter*

6.2. Comparison of the critical concentration for *L. pneumophila* with the fecal pathogens

As guidance values given in the Dutch legislation are to address health risks of fecal pathogens, the mean critical concentration of *L. pneumophila* was compared to the critical concentration estimated for the index fecal pathogens as given in Figure 11 and are interpreted from Table 4: compared to the fecal index pathogens, the critical concentration of *L. pneumophila* is six to seven log units higher.

Opposed to the ingestion of drinking water, infections with *L. pneumophila* occur due to inhalation of bioaerosols containing cells of *L. pneumophila.* A mean inhaled volume of 89.1 liters leads to a critical concentration of 2.2×10^{-4} CFU/L in the air, providing profound insight the exposure due to inhalation of *L. pneumophila* patently results in a lower health risk than the risk stemming from the exposure via ingestion of fecal pathogens. As the risk is attenuated by the aerosolization processes, the risk decreases with the exposure route via inhalation instead of a decreasing from risk of the *Legionella* dose in drinking water itself.

Concerning the risk from fecal pathogens, the critical dose of organisms to give a health response [CFU/d] equals the critical concentration in water [CFU/L]. This analogy is the result of one liter of tap water ingested per person per day being the premise of the estimation.

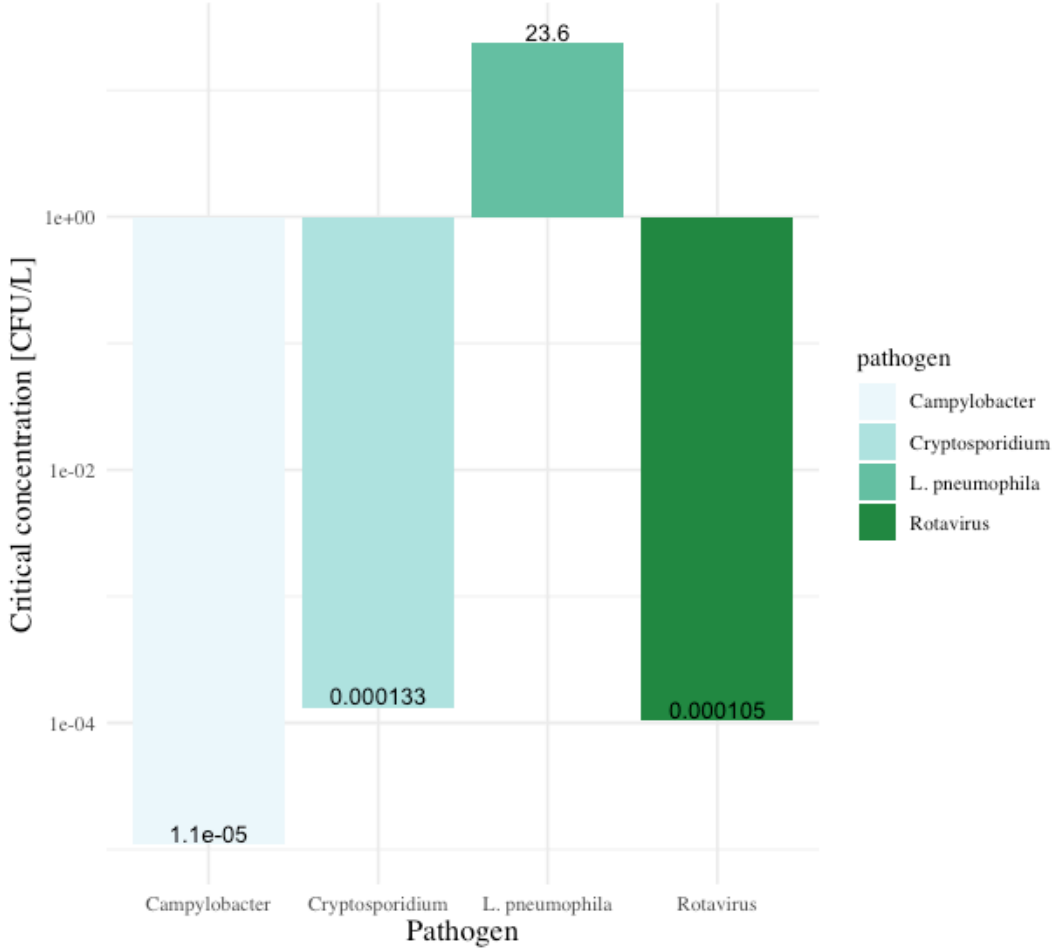

Figure 11. *The critical concentration for fecal pathogens and L. pneumophila estimated with the reverse QMRA method and the DALY as starting point; f_s and $P_{ill|inf}$ = 100%*

Table 4. The critical concentration of fecal pathogens and L. pneumophila estimated with the reverse QMRA method and the DALY as starting point, f_s = 100%

	Cryptosporidium	**Campylobacter**	**Rotavirus**	***L. pneumophila***
Dose of organisms per day (CFU/d)	1.33×10^{-4}	1.05×10^{-4}	1.11×10^{-5}	1.84×10^{-2}
Exposure route[1]	ingestion	ingestion	ingestion	inhalation
Critical concentration in water (CFU/L) [mean]	1.33×10^{-4}	1.05×10^{-4}	1.11×10^{-5}	23.6
Critical concentration in the air (CFU/L) [mean]	Not applicable	Not applicable	Not applicable	2.22×10^{-4}

1 *Exposure due to the exposure of ingestion of one litre of tap water*

6.3. The Spearman correlation analysis of the inhalation rate, shower time and partition coefficient with the critical concentration

To determine the influence of the model input parameters inhalation rate, shower time and partition coefficient on the uncertainty and variability of the critical concentration for *L. pneumophila* in drinking water, the Spearman rank correlation test was conducted. The results in Figure 12 demonstrate that of the three tested variables the partition coefficient has the highest Spearman correlation coefficient (-0.81) and is thus the dominant factor in terms of correlation with the critical concentration of *L. pneumophila* in drinking water. Distinct evidence for this conclusion is supported by the findings of Deans et al. (2020), showing that there is a strong negative correlation implying a diminution in critical concentration for an increasing PC_{bwa} (Figure 7.2). More than that, the critical concentration of *L. pneumophila* should be interpreted with caution in respect to the variation in PC_{bwa}, as the air-to-water partition is a subject of limited available knowledge (Chattopadhyay's (2017).

The Spearman correlation coefficient for the shower time is -0.09, which indicates a very weak and neglectable influence of the shower time on the critical concentration of *L. pneumophila*. In other words, a shorter or longer shower event has no crucial effect on the risk of contracting Legionnaires' disease. Due to this very low effect of the shower time, the uncertainty in shower time distribution (imprecise reported shower time duration by survey subjects) is trivial. This can be explained with the relatively small range of the distribution chosen, as the values are closer to the mean shower time.

For the correlation of the variability in inhalation rate, a negative moderate correlation (-0.54) has been observed with the critical concentration of *L. pneumophila* in drinking water, implying that the critical concentration is considerably influenced by the distinctive human anatomy of the inhalation rate. The domination of the inhalation rate over the shower time can be explained with the larger variation of the inhalation rate distribution used.

The variability of the observed inhalation rate was studied, where age was a factor under observation to determine the variability in inhalation rate. For this reason, the uncertainty of the inhalation rate is low and the variability high, with a low degree of uncertainty within the variability (USEPA, 2011).

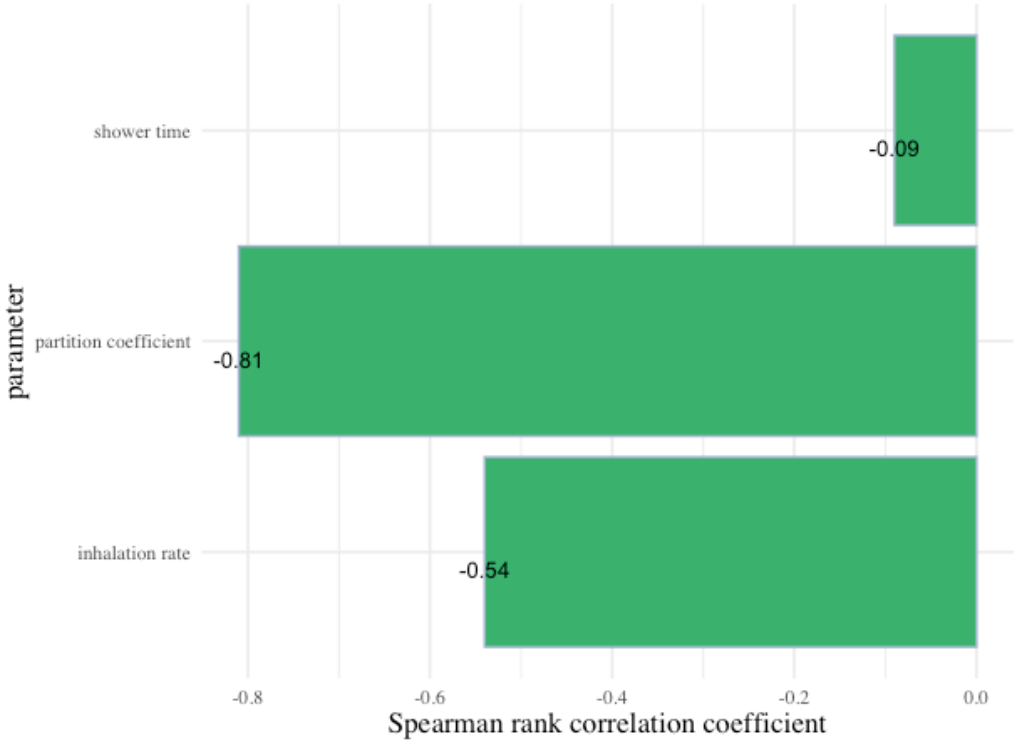

Figure 12. *Results of the correlation analysis showing the weight of the input parameter distribution with variability on the result of the critical concentration in water*

7. Discussion: Risk analysis of the critical concentration for *L. pneumophila*

7.1. Performance of the reverse QMRA in context of preliminary risk assessments on *L. pneumophila*

L. pneumophila remains a topic on the agenda of public health, as risk managers and scientists alike were heretofore unable to reach consensus on its risk status. This becomes evident from the diverging guidance values for *L. pneumophila* mandated by different health authorities around the world (Hamilton et al., 2019).

The reverse QMRA is a novel model as there were only two similar studies conducted by Rasheduzzaman (2019) and Hamilton et al. (2019) before. The results from this study show that the critical concentration for *L. pneumophila* in drinking water estimated with the 10^{-6} DALY approach is two log units lower than the critical concentration estimated with the 10^{-4} infection risk approach. Rasheduzzaman et al. (2019) also concluded from their study on reverse QMRA as a decision support tool, that the critical concentration of *Pseudomonas aeruginosa* was lower using a 10^{-6} DALY target than a 10^{-4} infection risk target. Hence, the utility of the approach was validated as such (although Rasheduzzaman did not report on *L. pneumophila*). For this purpose, the critical concentration for *L. pneumophila* was compared to the results of Hamilton's (2019) study on reverse QMRA for *L. pneumophila* (Table 5).

Table 5. The comparison of the critical concentration for L. pneumophila of this study with the results given by peer sciences

Hamilton et al., (2019) [CFU/L]	**Study's results (2021) [CFU/L]**	**Notes to Hamilton et al., (2019)**
12.3	23.6 (7.9 – 105.1)	DALY; mean of conventional fixtures*
48.1	23.6 (7.9 – 105.1)	DALY; mean water efficient fixtures*
14.4	23.6 (7.9 – 105.1)	Per-exposure-corrected DALY target for showers
1400	1963.6	Infection risk: Single sample shower
0.01 – 1	23.6 (7.9 – 105.1)	CSI+; severe case with hospitalization
10 – 1000	1.6 – 9.6	Immunocompromised fraction is compared to the critical concentration estimated for Pontiac fever from Hamilton

*The median critical concentration combines faucets of showers, toilets, and taps

+Clinical severity infection

Hamilton et al. (2019) used the same DB value of 0.97 DALY in their QMRA model for *L. pneumophila* but discerns between clinical (legionellosis) and non-clinical (Pontiac fever)

infections as well as conventional and water-efficient fixtures. Conforming to his results, the mean critical concentration corrected with the DALY target for showers is 14.4 CFU/L and is similar to the mean critical concentration estimated in this study with the DALY target for showers (23.6 CFU/L). The critical concentration for *L. pneumophila* estimated with the infection risk approach of this study is approximately 25% (564 CFU/L) higher than Hamilton's estimation. The most reasonable explanation why the mean critical concentration calculated in this study differs from Hamilton's results is that the variability of the PC_{bwa} used in the reverse QMRA differed between this and Hamilton's study. The difference was due to the aerosol size profile he used instead of the ratio of *L. pneumophila* observed in water and air. A second explanation for the difference between the studies may be structural uncertainties of the Monte Carlo algorithm (subsection 7.4.1).

Opposed to the results of Hamilton, the critical concentration estimated in this study for the immunocompromised is lower (1.6 – 9.6 CFU/L) compared to the concentration estimated for the whole population (7.9 – 105.1 CFU/L). This implies that a smaller susceptible population fraction addressed the risk for the immunocompromised correctly, as a large fraction of the population is assumed to be non-susceptible. Hamilton's finding yielded a higher critical concentration for Pontiac fever than for CSI's (clinical severity infection), for which she relates the latter result to the immunocompromised fraction. The difference in result is delineated with the difference in methodology and its underlining assumptions. This ultimately raises the question, if it is legitimate to allow for a higher critical concentration solely on the perception that the fraction of people subject to a higher chance of an infection with *L. pneumophila* is only 7.1% or one in every 14 people. This is with the knowledge that western countries experience rapid aging of the population (Eurostat, 2021). Because potential protection measures for the immunocompromised might be difficult to implement in every-day situations where the non-immunocompromised make up the larger fraction, a stricter critical concentration would make sense in locations where the proportion of the immunocompromised is higher, such as health care facilities or retirement homes.

7.1.1. Factors not processed in the reverse QMRA model

While reverse QMRA-based studies are new to the scientific world, forward QMRAs have frequently been developed to estimate the health risk of *L. pneumophila*. Deviations from the

approach of this study compared to other models are delineated by the difference in parameter choice in the context of the model's emphasis chosen:
The fraction of biofilm, where *L. pneumophila* grows on, that is slough off from the premise piping surface by the shear stress from the water flow was given consideration by Schoen and Ashbolt (2011) in their forward QMRA model on the infection risk of *L. pneumophila*.

Secondly, the aerodynamic particle size and their behavior in relation to aerosolized pathogens has been subject to various research attempts (Hung et al., 2020; Hamilton et al., 2019), translating the motion and partition through the air into a model. This leads to the manifold development and application of partitioning coefficients varying in their log order. For this study, a partitioning coefficient was chosen that was based on measured difference between the pathogenic concentration in the air and water of a controlled shower room (Chattopadhyay et al., 2017). A more comprehensive discussion on the air-to-water partition of bioaerosols is given in subsection 7.4.2.

7.2. The critical concentration and the Dutch Drinking Water Act

Based on the different health target set as starting point of the reverse QMRA, the critical concentration becomes either more conservative for a stricter target or less conservative for a more lenient target. While the mandated target is the infection risk of 10^{-4} in the *Dutch Drinking Water Act (2011),* the more conservative WHO proposed target of 10^{-6} DALY can supposably lead to proper health intervention for the disease burden incurred by opportunistic pathogens (Schijven et al., 2011). Nonetheless, it remains debatable if enteric and opportunistic pathogens should have different targets, and, even more so if a more conservative target would indeed reduce the number of *Legionella* infections, which is the ultimate goal of this risk assessments.

The minimum (7.9 CFU/L) and mean (23.6 CFU/L) critical concentration of *L. pneumophila* estimated with the 10^{-6} DALY target are both notably below the 100 CFU/L *Legionella spp.* mandated by the *Dutch Drinking Water Act* and the parametric value of 1000 CFU/L *Legionella spp*. for the new *European Drinking Water Directive* (Schijven et al., 20011; WHO, 2017). This is not the case for the critical concentration estimated with the infection risk approach, were the minimum and the mean critical concentration are larger (680 and 1963.6 CFU/L).

As a result of the more conservative DALY approach for *L. pneumophila*, the insufficiency of the target mandated by the *Dutch Drinking Water Act* and the *European Drinking Water Directive* is apparent, as it becomes evident that with the current target, the number fatalities (approximately 40 reported cases annually) cannot be prevented (Bijkerk et al., 2014). Further, the severity of a disease caused by an opportunistic pathogen (in this case *L. pneumophila*) cannot be adequately quantified with the infection risk approach, as the estimated minimum critical concentration is not lower than the mandated target, while the reported number of deaths remain steady.

Under subsection 6.1.2, a third critical concentration was introduced, that was derived from the DALY target for *Campylobacter* that corresponds to the infection risk target of 10^{-4} for *Campylobacter.* Because the estimated critical concentration for *L. pneumophila* using that approach (3.3 CFU/L) also corresponds to the legislative health target of 10^{-4} mandated by the Dutch government and the health risk of the fecal pathogen *Campylobacter*, the DALY method should lead to acceptance of the risk of *L. pneumophila* by the Dutch government as well.

While the ineffectiveness of the current guidelines in the Dutch Drinking Water Act has been contested, the deficiencies of the reverse QMRA developed for this study will be discussed in subsection 7.4. in terms of the reliability of the critical concentration estimated for *L. pneumophila.* Once these have been addressed and improvements have been made accordingly, the mandated allowable concentration for *L. pneumophila* can be aligned with the results of the reverse QMRA.

7.3. The critical concentration and the concentration of *L. pneumophila* detected in Dutch drinking water

The minimum, maximum, and mean (for f_s=100%) critical concentration for *L. pneumophila* for both the 10^{-6} DALY and the 10^{-4} infection risk target were compared to the measured concentration in Dutch drinking water in Table 6. Research in the Netherlands surveying single-family houses (n =400), reported 4 to 26% of *Legionella ssp* positive samples in Dutch drinking water, based on the minimum concentration of 100 CFU/L and higher (van der Kooij, 2013).

The maximum measured concentration of 10,000 CFU/L is much higher than the critical concentration estimated with the DALY, exhibiting more affirmation, that the health risk caused by *L. pneumophila* is not adequately addressed. The mean critical concentration estimated for the smallest susceptibility fraction (f_s= 7.1% for age 50 and male) and with the infection risk approach also exceeds the measured maximum concentration of *L. pneumophila* in Dutch drinking water. This points towards the attention needed for people considered immunocompromised. While the minimum critical concentration for *L. pneumophila* of 7.9 CFU/L conforms the health target of 10^{-6} DALY, there is a 4 to 26% chance, that this critical concentration is exceeded (van der Kooij, 2013).

Table 6. Comparison of the critical concentration of L. pneumophila to the observed critical concentration of Legionella spp in Dutch drinking water measured at the tap

	Measured in Dutch drinking water [CFU/L] (van der Kooij, 2013)	**Critical concentration with the DALY [CFU/L]**	**Critical concentration with the infection risk [CFU/L]**
Minimum	<100	7.9	680.8
Maximum	10,000	105.1	10,139.8
f_s = 7.1%	-	1.6	157.8

7.4. Interpreting the underlying assumptions of the parameters

7.4.1. Limitations of the Monte Carlo algorithm

Structural uncertainties caused by the statistical simulation caused by the Monte Carlo algorithm and are a widely acknowledged occurrence in QMRA, causing uncertainty in the critical concentration. These structural uncertainties must be accepted for the purpose of a simplified, modelled reality.

Aleatoric uncertainties are observed, as the random sample drawn from the impute distribution of the three parameters remains inconclusive as these differ with every statistical run. The dimensionality is amplified by the multivariate of the critical concentration, as the number of variables increases the range in critical concentration, providing an unknown result instead of one distinctive answer. The multivariate issue is enhanced by the unidentifiability of the multiple random combination of unknown parameters, which leads to a critical concentration that cannot distinguish between the parameters underpinning the critical concentration.

While the inhalation rate, the shower time and the partition coefficient have been processed as distributions, this has not been the case for the risk of illness given infection or the dose-response parameter. Translating these factors into a distribution would increase the insight in the range of possible outcomes in critical concentration. In addition, a correlation analysis could be conducted, showing how strong the influence of these additional distributions is on the estimated critical concentration of *L. pneumophila*. With this in mind, the Monte Carlo simulation would enhance the reliability of the reverse QMRA needed to yield the accurate critical concentration for *L. pneumophila*.

7.4.2. Modelling the air-to-water partition of bioaerosols

The estimation of the critical concentration for *L. pneumophila* points toward the route via inhalation and, that at this point, the critical concentration of *L. pneumophila* present in the air of the shower room demonstrates concerning values for the mean allowable respirable concentration in the air inhaled (2.2×10^{-4} CFU/L/d).

The nature of aerosol partitioning has been subject to various modelling attempts, underpinning the scientific relevance to capture the interaction of the water-air-inhalation-route on the one hand, and on how little the underlying mechanisms are understood on the other. According to the correlation analysis of the QMRA model for *L. pneumophila* by others (Schoen & Ashbolt 2011; Dean et al., 2020) and this study, the partitioning coefficient shows the highest influence on the concentration of *L. pneumophila* in water, and into which direction research can be funneled to, as finding the accurate partitioning coefficient parameter has proven to be challenging.

The partitioning coefficient of *L. pneumophila* has been estimated by Huang et al., (2019), who simulated a shower room of 6 m^3 to estimate this parameter. Their approach demonstrates the variability of the aerosol generation rate and the aerosol size distribution. Huang et al., (2019) further emphasizes, that viable but not culturable cells (VBNC) were not considered due to the lack of data of these cells being aerosolized into the air. If research was able to proof that VBNC *L. pneumophila* were present in the shower room air and be able to cause infection via the inhalation route, then the critical concentration estimated through this reverse QMRA model would become more conservative. Building on this assumption, it is uncertain whether the room size influences the partition of water to air molecules. Further, there has been no

indication in peer studies whether open or closed windows and doors influence the partition coefficient in terms of the aerosol removal rate. Another potential important factor that affects the partition of water to air molecules is the design of the shower head, but the influence of shower head design on this partition has not been studied beforehand.

As concluded in Chattopadhyay's (2017) study, the flow rate of the shower faucets is the most influential factor in terms of partitioning. Therefore, it is suggested to study the influence of the flow rate through the shower head on the partitioning coefficient and the critical concentration. Such research could be combined with research onto the influence of the shower head design on the partition of air molecules. Based on these findings, research can advise on engineering measures such as low risk shower head installations, avoid extreme water saving showers that reduce demand and simultaneously increase the residence time of water in the in-house plumbing, in health care facilities accordingly.

For the estimation of the allowable respirable concentration, the total volume inhaled during a shower event was computed. The average inhalation rate was considered, however different breathing patterns (nasal or oral inhalation) and inhalation rate demonstrate the variability on a person's physiological characteristics and lifestyle. The variability is a matter of behavioural patterns, directly affects the volume inhaled during one exposure event. While the average shower time has been determined, no study addressed the uncertainty if aerosols staying in the air after the shower event have an effect. If so, does this affect the proportion of people remaining in the bathroom after the actual shower event – increasing the exposure time –, or does the aerosol removal rate reduce the aerosols adequately immediately after closing the tap?

The aggregation of aerosols into the air of the shower room has been researched by Chattopadhyay et al. (2017), who determined that the rise time to generate aerosols is 4.5 minutes, which means that 90% of the maximum aerosol concentration has been reached inside the shower cabin after 4.5 minutes. As the critical concentration reaches its maximum at this point, it can be assumed that a deferral of the time to generate aerosols could lead to a risk reduction. However, it remains unclear which fraction of aerosolized *L. pneumophila* cells can infect a host. This means that the fragile nature of viable cells depends on relative humidity, and which is not quantified in the aerosolization process, but has been proven to decrease with low humidity as the probability of cell stress enhances causing cell desiccation (Chattopadhyay et al., 2017). If the humidity increases with time and it is assumed to coincide with the time of

4.5 minutes to generate aerosols, then it is recommended to study the relationship between open windows (mechanic ventilation) and the aerosol removal rate and how this influences the critical concentration in air and water.

The fraction of aerosolized *L. pneumophila* partitioning into air depends on the size range of aerosols, essentially determining the fraction deposition in the lower respiratory system. As a final limitation, aerosol sampling devices as much as the lack of protocols on aerosol sampling impede scientific investigations for this matter and do greatly disfigure the ratio of the partition coefficient. Restricting factors causing inefficiency of the measuring devices are low sampling rates, fixed shower head flow rates, a limited sampling time due to evaporation and liquid impingement reducing data collection on particle size (Chattopadhyay et al., 2017).

7.4.3. The probability of infection given illness and the dose-response

With reference to the probability of an infection, this study directs research towards the prevalence of antibodies against *L. pneumophila* within a chosen population, that are extrapolated for the estimation of the probability of illness given infection. The assumption is that not every infection with *L. pneumophila* develops into clinical symptoms observed for legionellosis. Pertinent to the method of this study is that antibody levels in humans are high enough to provide immunity for a year and that immunity is developed through the average dose per exposure event. Further, immunity was assumed to be lower in the population fraction assigned with a susceptibility fraction, which yields a ratio of reported illness against the population. The actual ratio of infection with *L. pneumophila* to legionellosis infections is uncertain (Annex II). As there has been no data available on the probability of illness given infection of *L. pneumophila* in the WHO study on *QMRA Applications for Water Safety Management*, research towards understanding this probability of illness given infection is recommended in order understand the correlation between the risk of infection and the risk of illness given infection. With this in mind, data on the correlation between the risk of illness given infection and the critical concentration *of L. pneumophila* can may be beneficial towards designing a more reliable reverse QMRA model for *L. pneumophila*.

As there has no distinction been drawn between the immunocompromised and the immunocompetent, the (reverse) QMRA would also benefit from better understanding on microbiological data on the dose-response mechanisms of immunocompromised people. It is plausible, that an immunocompromised person is likely to contract an illness by *L.*

pneumophila at a lower dose of exposure. The dose-response is a knowledge gap in the field of infectious diseases and in need of more data but studies to determine this factor are difficult to perform due to moral acceptance on population or animal studies. An alternative option are in vitro studies, which have not been developed yet for *L. pneumophila* (Goh et al., 2015).

7.4.4. The Disease Burden

Drawing the critical concentration from the DALY yields a more stringent result compared to the model with the infection risk approach, indicating the significance of the disease burden as component in risk characterization of waterborne pathogenesis, and leading to the manifestation of the DALY as alternative fundamentally. Whether or not the DALY and the critical concentration should be used as a guideline for a waterborne pathogen remains questionable (Table 7): for an opportunistic infection such as Legionnaires' disease, the disease burden as starting point allows for a better comparison for infectious diseases with a high disease burden and a low case number. This, however, might not be the case for fecal pathogens, where the disease burden is low, but the number of observed cases annually is high. A disease-causing pathogen with a relatively high death number can – opposed to gastroenteritis – lead to societal unrest, e.g., after the *Bovenkarspel legionellosis outbreak*, raising the question if public health interventions direct their attention to the right pathogen(s) (Den Boer et al., 2015).

Table 7. Comparison of various waterborne fecal- and opportunistic diseases, with number of annual cases observed and the DALY pppa attributed to the disease burden (Bijkerk et al., 2014).

Illness	**DALY [pppa]**	**Number of cases [annually]**	**Critical concentration [CFU/L]**
Cryptosporidiosis	0.0015	184	1.33×10^{-4}
Campylobacter	0.0046	8547	1.05×10^{-4}
Salmonellosis	0.003	2029	-
Legionnaires' disease	0.97	312	23.6

The critical concentration estimated for *L. pneumophila* was calculated using the DALY approach with the aim to incorporate the disease burden of Legionnaires' disease instead of the number of infections reported only. Within the DALY framework and for the purpose of the health risk assessment, the sequalae for an infection with *L. pneumophila* can range from a self-limited flue like course of the infection to severe pneumonia and death. The individual course

of disease is due to its heterogeneity difficult to link to the dose-response in humans, especially in context of the susceptibility of one's immunity (Bentham et al., 2018). Because a lower dose-response parameter than fixed value used in this study (k = 0.059), which models the relation between the number of organisms needed to cause an infection, this value might not be applicable for an immunocompromised person, who's risk of infection would occur at a lower dose. In this case a lower dose-response parameter ($k = 6.48 \times 10^{-5}$) might be more applicable (Fitzgeorge et al., 1983). Hence, using a fixed, best-fit, dose-response parameter distorts the outcome of the reverse QMRA, whereas a distribution might lead to a better outcome.

Bentham et al., (2018) argues, that the DALY for *L. pneumophila* underlines the YLL as being the dominant component of the disease burden (92%). When comparing the weight of the YLL (3892) to the weight of the YLD (391), then it becomes apparent, that the mortality outweights the morbidity, where one could argue that an adjusted dose-fitting parameter is imperative to model YLL accurately (Bijkerk.et al., 2014). An alternative approach is to differentiate between the YLD and YLL, where YLL is remodeled for a lower dose-fitting parameter as reasoned above.

As for the YLD, disability weights are allocated with a disability weight rated from 0 – 1, a concept developed by a panel of medical professionals that use 16 *indicator conditions* and their descriptions as reference for the weighting of other diseases. As addition to the standard health state description, the EuroQol 5D classification describes six generic dimensions – mobility, self-care, ability to perform usual activities, pain/discomfort, anxiety/depression, and cognition – divided into three levels – no problems, moderate problems, and severe problems. This approach makes the quantification of the disease burden questionable, as one could argue that a DW heavily relies on finding a panel of experts that is qualified enough to reach consensus on finding one number that describes DW sufficiently accurate (Stouthard et al., 1997).

Monitoring of the annual number of legionellosis cases is recommended, as these have increased, as reported by the RIVM in September 2021. As a result of the increased reported number of cases, the DB of *L. pneumophila* has increased from 0.97 to 1.1, causing the mean critical concentration to drop from 23.6 CFU/L to 20.8 CFU/L (Reukers et al., 2020). The updated DB has, thus, no crucial influence on the outcome of the results of this study.

7.5. The health paradigm of *L. pneumophila*

The implication whether the disease burden of *L. pneumophila*, and opportunistic pathogens in general, is detrimental enough to be under public health revision, is not only a matter of environmental health ethics but also a question whether or not Dutch health authorities are willing to pioneer towards addressing health impacts in manner that addressed the severity of a disease rather than the infection alone

In terms of public risk policy and risk acceptance, statistical tabulations comparing one risk to another have been considered a powerful tool for distortion and rejection by shifting the perspective. The absolute numbers, – mortality and morbidity – despite the nature on how a risk is communicated, does remain the same (Mayo and Hollander, 1991). Comparison of risks is indistinguishable from placing one unlikely negative hazard next to another, which appear to trivialize the risk – of a waterborne disease – through preconception of cross-hazard risks comparison (Covello et al., 1991). In the *Guidelines for communicating information about chemical risks effectively and responsibly,* Covello et al., *(1991)* argues, that an unlikely risk should be compared to an unlikely positive event. For this, the following scenario is suggested from this study:

One faces the possibility to be the acquirer of newly built real estate property in a highly competitive neighborhood. Further, his/her change is equal to the chance of catching Legionnaires' disease from a shower event. It can relatively easy be assumed that one easier "believes" and hence accepts the chance of becoming the new owner of the property, even if that possibility is low, than that accepts the chance of an unlikely risk hazard. While the first scenario is a positive unlikely possibility, the other is a negative possibility. The chance for either one does not change and is equally low.

The benefits of accepting the evidence of the risk and its measures can easily be described as that a stricter *Drinking Water Act with a target* of 10^{-6} DALYs and a maximum concentration of 7.9 CFU/L *L. pneumophila* could save on average 36 lives per year, prevent on average 881.4 infections leading to *Legionella* pneumonia (as reported by the RIVM), and reduce the incurred medical costs of approximately 37,300 USD per hospitalization with Legionnaires' disease (Bijkerk et al., 2014; Collier et al., 2021). In order to accomplish this goal and to ensure that the maximum allowable concentration for *L. pneumophila* is effective, other sources than drinking water (such as cooling towers or wastewater treatment plants) must be eliminated, as the effect on the *Legionella* cases will otherwise be redundant.

8. Monitoring of *L. pneumophila* in the future

8.1. Conclusion

The risk characterization of *L. pneumophila* showed a large difference in critical concentration between a 10^{-6} DALY and a 10^{-4} infection risk target. While the critical concentration for *L. pneumophila* in drinking water is in line with the *Dutch Drinking Water Act* for the 10^{-4} infection risk target, this is not the case for the 10^{-6} DALY target, where a mean critical concentration of 23.6 CFU/L is lower than the mandated 100 CFU/L, validating the risk as such.

The critical concentration estimated for the vulnerable subgroups of the Dutch population yielded slightly lower values, with a mean critical concentration of 1.6 – 9.6 CFU/L (depending on the subgroup) and estimated with the 10^{-6} DALY target.

The critical concentration of fecal pathogens is several log units lower compared to the critical concentration for *L. pneumophila*, however the their critical concentration lie several log units beyond the detection limit.

8.2. Recommendations

The reverse QMRA together with the DALY is recommended for the risk assessment of waterborne disease transmission as this method more accurately assesses the risk for opportunistic waterborne diseases that have a high disease severity that with the conventional infection risk approach is not addressed. More so, the research outcome endorses risk assessments with the health quantification of the DALY method for atypical pathogenesis for the group of various opportunistic pathogens, especially considering the relatively high fatality rate.

In order to ensure that the estimated allowable critical concentration for *L. pneumophila* is effective in terms of a disease burden reduction, the reverse QMRA together with the 10^{-6} DALY target can be repeated for exposure routes besides showering and drinking water.

Because the critical concentration is exceeded by the *Legionella* concentration present in drinking water at certain places, the risk is higher than the 10^{-6} DALY. While the number of

cases is still deemed low, decision makers accept the risk being present and taking cognizance of the strong limitations to assess the probability of contracting Legionnaires' disease that come with that decision. For this purpose, research towards designing a more robust model is recommended. Addressing the air-to-water partitioning coefficient, the risk of illness given infection, the dose-response of immunocompromised people, the disability weight quantification in terms of '*what is the state of compromised health*', would be a merit to reduce waterborne disease transmission.

References

[1] Armstrong, T.W. and Haas, C.N., 2007. A quantitative microbial risk assessment model for Legionnaires' disease: Animal model selection and dose-response modeling. *Risk Analysis: An International Journal*, *27*(6), pp.1581-1596.

[2] Benke, K.K. and Hamilton, A.J., 2008. Quantitative microbial risk assessment: uncertainty and measures of central tendency for skewed distributions. *Stochastic Environmental Research and Risk Assessment*, *22*(4), pp.533-539.

[3] Bentham R & Whiley H (2018) Quantitative Microbial Risk Assessment and Opportunist Waterborne Infections–Are There Too Many Gaps to Fill? *International Journal of Environmental Research and Public Health* **15**.

[4] Bijkerk, P., van Lier, A., McDonald, S., Kardamanidis, K., Fanoy, E.B., Wallinga, J., de Melker, H.E., 2014. *State of infectious diseases in the Netherlands, 2013.* RIVM. ISSN: 1875-0885

[5] Bijkerk, P., van Lier, A., McDonald, S., Wallinga, J. and de Melker, H.E., Appendix: State of infectious diseases in the Netherlands, 2013. Bilthoven: National Institute for Public Health and the Environment (RIVM); 2014 (Appendix RIVM report 150205001).

[6] Bosch, A., 2007. *Human viruses in water: Perspectives in medical virology*. Elsevier.

[7] Chattopadhyay, S., Perkins, S.D., Shaw, M. and Nichols, T.L., 2017. Evaluation of exposure to Brevundimonas diminuta and Pseudomonas aeruginosa during showering. *Journal of aerosol science*, *114*, pp.77-93.

[8] Collier, S.A., Deng, L., Adam, E.A., Benedict, K.M., Beshearse, E.M., Blackstock, A.J., Bruce, B.B., Derado, G., Edens, C., Fullerton, K.E. and Gargano, J.W., 2021. Estimate of burden and direct healthcare cost of infectious waterborne disease in the United States. *Emerging infectious diseases*, *27*(1), p.140.

[9] Corvalan C., Mathers C., Prüss-Üstün A., Woodward Alistair., 2003. *Assessing the environmental burden of diseases at national and local levels*. World Health Organization Protection of the Human Environment Geneva.

[10] Covello, V.T., Sandman, P.M. and Slovic, P., 1991. Guidelines for communicating information about chemical risks effectively and responsibly. *Acceptable evidence: Science and values in risk management*, pp.66-90.

[11] Dean K & Mitchell J (2020) A dose response model for the inhalation route of exposure to P. aeruginosa. *Microbial Risk Analysis* **15**: 100115.

[12] Dean, K. and Mitchell, J., 2020. Reverse QMRA for Pseudomonas aeruginosa in premise plumbing to inform risk management. *Journal of Environmental Engineering*, *146*(3), p.04019120.

[13] Den Boer, J.W., Euser, S.M., Brandsema, P., Reijnen, L. and Bruin, J.P., 2015. Results from the National Legionella outbreak detection program, the Netherlands, 2002–2012. *Emerging infectious diseases*, *21*(7), p.1167.

[14] Drago, L. and Toscano, M., 2017. Biofilm formation and the biological response. In *Management of Periprosthetic Joint Infections (PJIs)* (pp. 25-39). Woodhead Publishing.

[15] Eurostat (2021) *Population structure and aging*. Available at: https://ec.europa.eu/eurostat/statistics-explained/index.php?title=Population_structure_and_ageing

[16] Feigin, V.L., Roth, G.A., Naghavi, M., Parmar, P., Krishnamurthi, R., Chugh, S., Mensah, G.A., Norrving, B., Shiue, I., Ng, M. and Estep, K., 2016. Global burden of stroke and risk factors in 188 countries, during 1990–2013: a systematic analysis for the Global Burden of Disease Study 2013. *The Lancet Neurology*, *15*(9), pp.913-924.

[17] Fitzgeorge, R.B., Baskerville, A., Broster, M., Hambleton, P. and Dennis, P.J., 1983. Aerosol infection of animals with strains of Legionella pneumophila of different virulence: comparison with intraperitoneal and intranasal routes of infection. *Epidemiology & Infection*, *90*(1), pp.81-89.

[18] Goh, J.Y., Weaver, R.J., Dixon, L., Platt, N.J. and Roberts, R.A., 2015. Development and use of in vitro alternatives to animal testing by the pharmaceutical industry 1980–2013. *Toxicology Research*, *4*(5), pp.1297-1307.

[19] Hamilton, K.A., Hamilton, M.T., Johnson, W., Jjemba, P., Bukhari, Z., LeChevallier, M. an Haas, C.N., 2018. Health risks from exposure to Legionella in reclaimed water aerosols: Toilet flushing, spray irrigation, and cooling towers. *Water research*, *134*, pp.261-279.

[20] Hamilton, K.A., Hamilton, M.T., Johnson, W., Jjemba, P., Bukhari, Z., LeChevallier, M., Haas, C.N. and Gurian, P.L., 2019. Risk-based critical concentrations of Legionella pneumophila for indoor residential water uses. *Environmental science & technology*, *53*(8), pp.4528-4541.

[21] Havelaar, A.H. and Melse, J.M., 2003. Quantifying public health risk in the WHO Guidelines for drinking-water quality: A burden of disease approach

[22] Havelaar, A.H. and Swart, A.N., 2014. Impact of acquired immunity and dose-dependent probability of illness on quantitative microbial risk assessment. *Risk Analysis*, *34*(10), pp.1807-1819.

[23] Hofbauer, W.K., 2018. Biofilme mit Amöben, Bakterien und Pilzen im gebauten Umfeld des Menschen. *Bauphysik*, *40*(5), pp.396-404

[24] Hsu, B.M., Huang, C.C., Chen, J.S., Chen, N.H. and Huang, J.T., 2011. Comparison of potentially pathogenic free-living amoeba hosts by Legionella spp. in substrate-associated biofilms and floating biofilms from spring environments. *Water research*, *45*(16), pp.5171-5183.

[25] Huang, C., Shen, Y., Smith, R.L., Dong, S. and Nguyen, T.H., 2020. Effect of disinfectant residuals on infection risks from Legionella pneumophila released by biofilms grown under simulated premise plumbing conditions. *Environment international*, *137*, p.105561.

[26] Kusumawardhana, A., Zlatanovic, L., Bosch, A. and van der Hoek, J.P., 2021. Microbiological Health Risk Assessment of Water Conservation Strategies: A Case Study in Amsterdam. *International Journal of Environmental Research and Public Health*, *18*(5), p.2595.

[27] Lu, J., Buse, H., Struewing, I., Zhao, A., Lytle, D. and Ashbolt, N., 2017. Annual variations and effects of temperature on Legionella spp. and other potential opportunistic pathogens in a bathroom. *Environmental Science and Pollution Research*, *24*(3), pp.2326-2336.

[28] Marszal, E.M., 2001. Tolerable risk guidelines. *Isa Transactions*, *40*(4), pp.391-399.
Meehl, G.A., Stocker, T.F., Collins, W.D., Friedlingstein, P., Gaye, A.T., Gregory, J.M., Kitoh, A., Knutti, R., Murphy, J.M., Noda, A. and Raper, S.C., 2007. Global climate projections. Chapter 10.

[29] Mayo, D.G. and Hollander, R.D. eds., 1991. *Acceptable evidence: Science and values in risk management*. Oxford University Press on Demand.

[30] Medema, G. and Ashbolt, N., 2006. QMRA: its value for risk management. *Comunidade Europeia*.

[31] Murray, C.J., Vos, T., Lozano, R., Naghavi, M., Flaxman, A.D., Michaud, C., Ezzati, M., Shibuya, K., Salomon, J.A., Abdalla, S. and Aboyans, V., 2012. Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990–2010: a systematic analysis for the Global Burden of Disease Study 2010. *The lancet*, *380*(9859), pp.2197-2223.

[32] National Academies of Sciences, Engineering, and Medicine, 2020. *Management of Legionella in water systems*. National Academies Press.

[33] National Research Council, 2009. Science and decisions: advancing risk assessment.

[34] Rasheduzzaman, M., Singh, R., Haas, C.N., Tolofari, D., Yassaghi, H., Hamilton, K.A., Yang, Z. and Gurian, P.L., 2019. Reverse QMRA as a decision support tool: Setting acceptable concentration limits for Pseudomonas aeruginosa and Naegleria fowleri. *Water*, *11*(9), p.1850.

[35] Reukers, D.F.M., van Asten, L., Brandsema, P.S., Dijkstra, F., Hendriksen, J.M.T., van der Hoek, W., Hooiveld, M., de Lange, M.M.A., Niessen, F., Teirlinck, A.C. and Meijer, A., 2021. Annual report Surveillance of influenza and other respiratory infections in the Netherlands: winter 2019/2020.

[36] Rijksinstituut voor Volksgezondheid en Milieu (2020) *Legionella.* Available at: https://www.rivm.nl/legionella

[37] Salomon, J.A., Haagsma, J.A., Davis, A., de Noordhout, C.M., Polinder, S., Havelaar, A.H., Cassini, A., Devleesschauwer, B., Kretzschmar, M., Speybroeck, N. and Murray, C.J., 2015. Disability weights for the Global Burden of Disease 2013 study. *The Lancet Global Health*, *3*(11), pp.e712-e723.

[38] Schijven, J.F., Teunis, P.F., Rutjes, S.A., Bouwknegt, M. and de Roda Husman, A.M., 2011. QMRAspot: a tool for quantitative microbial risk assessment from surface water to potable water. *Water research*, *45*(17), pp.5564-5576.

[39] Schoen, M.E. and Ashbolt, N.J., 2011. An in-premise model for Legionella exposure during showering events. *Water research*, *45*(18), pp.5826-5836.

[40] Seiler, C., van Velzen, E., Neu, T.R., Gaedke, U., Berendonk, T.U. and Weitere, M., 2017. Grazing resistance of bacterial biofilms: a matter of predators' feeding trait. *FEMS microbiology ecology*, *93*(9).

[41] Sphere Association, 2018 *The Sphere Handbook – Humanitarian Charter and Minimium Stondards in Humanitarian Response.*

[42] Stockhause, M., Juckes, M., Chen, R., Okia, W.M., Pirani, A., Waterfield, T., Xing, X. and Edmunds, R., 2019. Data Distribution Centre Support for the IPCC Sixth Assessment. *Data Science Journal*, *18*(1).

[43] Stouthard, M.E., Essink-Bot, M.L., Bonsel, G.J., Barendregt, J.J.M., Kramers, P.G., Van de Water, H.P.A., Gunning-Schepers, L.J. and van der Maas, P.J., 1997. Disability weights for diseases in the Netherlands.

[44] Trimbos Institute – Netherlands Expetise Centre for Tobacco Control (2018) *Smoking in the Netherlands: Key Statistics 2017. Available at: https://www.trimbos.nl/docs/9a7f5384-36fa-4edc-815f-1d0388960f46.pdf*

[45] USEPA. *Exposure Factors Handbook*; Washington, DC, 2011

[46] Van der Kooij P.W.J.J., 2013. Legionella in drinking-water supplies. *Microbial Growth in Drinking Water Supplies*, p.127.

[47] Van der Wielen, P.W.J.J., Italiaander, R., Wullings, B.A., Heijnen, L., Van der Kooij, D., van der Kooij, D. and van der Wielen, P.W.J.J., 2013. Opportunistic pathogens in drinking water in the Netherlands. *Microbial Growth in Drinking Water Supplies*, p.177.

[48] Van der Wielen, P. W. J. J.; Wullings, B. A. In *Genotype analyses of Pseudomonas aeruginosa, Stenotrophomonas maltophilia and Aspergillus fumigatus isolates from drinking water reveal similar genotypes with patient strains.*, 20th International Symposium on Health Related Water Microbiology, Vienna, Austria, IWA: Vienna, Austria, 2019.

[49] van Leeuwen, C.J. and Vermeire, T.G. eds., 2007. *Risk assessment of chemicals: an introduction*. Springer Science & Business Media.

[50] Wang, H., Shen, Y., Hu, C., Xing, X. and Zhao, D., 2018. Sulfadiazine/ciprofloxacin promote opportunistic pathogens occurrence in bulk water of drinking water distribution systems. *Environmental Pollution*, *234*, pp.71-78.

[51] Weir MH, Mraz AL & Mitchell J (2020) An Advanced Risk Modeling Method to Estimate Legionellosis Risks Within a Diverse Population. *Water* **12**: 43.

[52] Word Health Organization, 2021. *Disability-adjusted life years (DALYs). Available at:* https://www.who.int/data/gho/indicator-metadata-registry/imr-details/158

[53] World Health Organization, 2017. Drinking Water Parameter Cooperation Project: Support to the revision of Annex I Council Directive 98/83/EC on the Quality of Water Intended for Human Consumption (Drinking Water Directive). *no. September*, pp.1-228.

[54] World Health Organization, 2011. *Evaluating household water treatment options: Health-based targets and microbiological performance specifications*. World Health Organization.

[55] World Health Organization, 2017. Guidelines for drinking-water quality: first addendum to the fourth edition.

[56] World Health Organization (2018) *Legionellosis.* Available at: https://www.who.int/news-room/fact-sheets/detail/legionellosis

[57] World Health Organization. (2016). Quantitative microbial risk assessment: application for water safety management.

[58] Xing, X., Li, T., Bi, Z., Qi, P., Li, Z., Wang, H., Lyu, L., Gao, Y. and Hu, C., 2021. Enhancing inhibition of disinfection byproducts formation and opportunistic pathogens growth during drinking water distribution by Fe2O3/Coconut shell activated carbon. *Environmental Pollution*, *268*, p.115838.

[59] Zhang, C. and Lu, J., 2021. Optimizing disinfectant residual dosage in engineered water systems to minimize the overall health risks of opportunistic pathogens and disinfection by-products. *Science of The Total Environment*, *770*, p.145356.

Supplementary Information

Annex I The susceptible fraction (f_s)

Annex II The probability of response for an infection

Annex III The risk of illness given infection

Annex IV The dose response

Annex V The health target derived from the DALY of the *Campylobacter* bacterium

Annex VI R-Script Code of the Monte Carlo Simulation

Annex I – The susceptible fraction (f_s)

Risk factors	**Absolute or relative number**	**Source**
Dutch population 50 years and older	7,061,393	Statistika
Dutch population over 50 smoking daily	17.2%	Trimbos
Dutch population 50 years and older being male	3,379,952	The world factbook
Total Dutch Population	17,167,263 (stand May 10th ,2021)	(Worldometers, 2021)

Risk Scenario 1. Dutch population 50 years and older and smoking daily

7,061,393 x 0,172 = 1,174,900

100/17,167,263*1,174,900 = 7.07%

Risk Scenario 2. Dutch population 50 years and older

100/17,167,263*7,061,393 = 41.13%

Risk Scenario 3. Dutch population 50 years and older being male

100/17,167,263* 3.379.952 = 19.7%

Annex II – The probability of response for an infection

The daily risk of infection (P.inf) was estimated for both the infection risk approach and the DALY as starting point, for which the estimation of P.inf of the DALY was estimated as exemplified below for the population fraction of 100%.

Annual probability of illness (P_{ill})

$$\frac{Health\ target}{(Daly/case_{pppa} \times f_s)} = \frac{10^{-6}}{0.97 \times f_s} = \underline{1.031 \times 10^{-6}}$$

where f_s = 100% = 1 for the total Dutch population. The variations in f_s are accounted for in the estimation of the risk of illness given infection. The factor estimated for the P_{ill} assumes that the probability of illness is equal throughout the population. Because f_s is accounted for the $P_{ill|inf}$ f_s equals 100% for P_{ill} as these factors would equalize each other.

Annual probability of infection ($P_{inf.ann}$)

$$\frac{P_{ill}}{P_{ill|inf}} = \frac{0.000001031}{0.0000026} = \underline{0.3905}$$

Daily risk of infection ($P_{inf.d}$)

$$\frac{P_{inf.ann}}{Number\ of\ exposure\ events/an.} = \frac{0.3905}{365} = \underline{1.07 \times 10^{-3}}$$

The annual probability for an infection ($P_{inf.ann}$) for the infection risk approach follows a similar procedure, where the Annual probability of illness (P_{ill}) corresponds with the risk target of 10^{-4}.

Annual probability of infection ($P_{inf.ann}$)

$$\frac{P_{ill}}{P_{ill|inf}} = \frac{10^{-4}}{0.0000026} = \underline{38.46}$$

Daily risk of infection ($P_{inf.d}$)

$$\frac{P_{inf.ann}}{Number\ of\ exposure\ events/an.} = \frac{0.71}{365} = \underline{1.05 \times 10^{-1}}$$

Annex III – The risk of illness given infection

The risk of illness given infection ($P_{ill|inf}$) was determined based on Havelaar's (2014) as portrayed as below:

$$\pi_{100\%} = \frac{916.6}{17{,}167{,}263 * 1 x\ 365\ x\ 0.0554} = \underline{0.000026}$$

$$\pi_{41.3} = \frac{916.6}{17{,}167{,}263 * 0.413 x\ 365\ x\ 0.0554} = \underline{0.0000064}$$

$$\pi_{19.7} = \frac{916.6}{17{,}167{,}263 * 0.197 x\ 365\ x\ 0.0554} = \underline{0.0000134}$$

$$\pi_{7.1} = \frac{916.6}{17{,}167{,}263 * 0.071 x\ 365\ x\ 0.0554} = \underline{0.0000377}$$

The median incidence number of *L. pneumophila* infections reported by the RIVM between 2007 and 2011 is 916.6 and was estimated from the median between the years 2007 and 2011 (Bijkerk, 2014). For the calculations with the infection risk target, the same susceptibility fractions were used.

$$I = \frac{Number\ of\ cases}{Number\ of\ years} = \frac{4583}{5} = \underline{916.6}$$

$P_{inf}(D)$ was estimated as below:

$$P_{inf}(1) = 1 - e^{-0.059\ x\ 1} = \underline{0.0554}$$

Annex IV – The dose response

The dose fitting parameter of the exponential dose-response model was used to calculate the allowable dose [CFU] of organisms per day (per shower event), where (1) indicates the allowable dose with the DALY as starting point and (2) with the infection risk as starting point. Both examples are estimated with a susceptibility fraction of 100%

$$\frac{\text{Pinf.d}}{dose-fitting\ parameter} = \frac{1.0863\ x\ 10^{-3}}{0.059} = \underline{1.8412 \text{ x } 10^{-2} \text{ CFU}} \quad (1)$$

$$\frac{\text{Pinf.d}}{dose-fitting\ parameter} = \frac{0.1053}{0.059} = \underline{1.7860 \text{ CFU}} \quad (2)$$

Annex V – The health target derived from the DALY of the *Campylobacter* bacterium

In a third approach the critical concentration for *L. pneumophila* was calculated with an alternative health target. For this approach the DALY of the *Campylobacter* bacterium was calculated, where P_{ill} was replaced with the infection risk target of 10^{-4}.

$$DALY_{Campylobacter} = P_{ill} \times db \times f_s \times 100 \quad (1)$$

$$DALY_{Campylobacter} = 10^{-4} \times 0.3 \times 0.00463 \times 100 \times 100 = \underline{1.39 \times 10^{-7}} \quad (2)$$

Annex VI – R Script Code of the Monte Carlo Simulation

```
Year <- 365
DALY_case <- 0.97
DALY_range <- runif(10000, min = 0.9, max = 1.05)
target <- 10^-6
risk.ill <- (10^-6)/(DALY_case*1)

#Pill.illness.given.infection
Pill.inf.100 <-0.0000026
Pill.inf.41 <-0.0000064
Pill.inf.19.7 <-0.0000134
Pill.inf.7.1 <- 0.0000377

#Daily risk of infection
risk.inf.d.100 <- risk.ill/Pill.inf.100/365
risk.inf.d.41.3 <- risk.ill/Pill.inf.41/365
risk.inf.d.19.7 <- risk.ill/Pill.inf.19.7/365
risk.inf.d.7.1 <- risk.ill/Pill.inf.7.1/365

#Dose-response-Cair
r <- 0.059 #dose-fitting-parameter

#Allowable dose of organisms inhaled per day
all.dose.100 <- risk.inf.d.100/r #allowable dose
all.dose.41.3 <- risk.inf.d.41.3/r #allowable dose
all.dose.19.7 <- risk.inf.d.19.7/r #allowable dose
all.dose.7.1 <- risk.inf.d.7.1/r #allowable dose

#shower time (4 - 14min, sd=0.4, lognormal)
set.seed(123)
shower_time <-rnorm(10000, mean = 7.8, sd = 0.4)  #Hamilton


summary(shower_time)

##    Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
##   6.262   7.533   7.796   7.799   8.069   9.339


#inhalation rate (min=0.0042, max=0.017, likelist=0.013)
library(triangle)
set.seed(123)
tri.IR <- rtriangle(10000, 0.0042, 0.017, 0.013)

#Volume: inhalation rate*shower time*1000
volume <-tri.IR*shower_time*1000

#Critical concentration in the air: dose/volume (4 different ones)
Cair.100 <- all.dose.100/volume
Cair.41.3 <- all.dose.41.3/volume
Cair.19.7 <- all.dose.19.7/volume
Cair.7.1 <- all.dose.7.1/volume
```

```
#partitioning coefficient (uniform distribution)
set.seed(123)
partitioning_coefficient <-runif(10000, min = 4.56*10^-6, max = 1.69*10^-5
)

#Critical concentration in water (Cair/PC) (4)
Cw.100 <- Cair.100/partitioning_coefficient
Cw.41.3 <- Cair.41.3/partitioning_coefficient
Cw.19.7 <- Cair.19.7/partitioning_coefficient
Cw.7.1 <- Cair.7.1/partitioning_coefficient

#min and max
summary(Cw.100) # 7.7 - 136.1

##    Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
##   7.598  12.841  19.005  26.308  32.405 125.134

summary(Cw.41.3)# 3.0 - 38.1

##    Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
##   3.087   5.217   7.721  10.688  13.164  50.836

summary(Cw.19.7)# 1.4 - 18.2

##    Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
##   1.474   2.492   3.688   5.105   6.287  24.280

summary(Cw.7.1)# 0.5 - 6.5

##    Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
##  0.5240  0.8856  1.3107  1.8143  2.2348  8.6299

#95% confidence #standard error sd(x)/sqrt(length(x))
SE.function <-function(x) sd(x)/sqrt(length(x))
SE.function(Cw.100) # 0.7062259

## [1] 0.1955931

standard_error <-0.7062259

#with a 95% confidence interval of (11.25297, 11.40301 CFU/L) for the DALY
metric
lb <- mean(Cw.100)-standard_error*qnorm(0.95) #lower bounds
ub <-mean(Cw.100)+standard_error*qnorm(0.95) # upper bounds
lb #(17.4)  #22.43

## [1] 25.14634

ub #(23.4) # 24.75  the mean should fall between those boundaries based on
the given population data distribution, with the actual value being unknow
n

## [1] 27.46962

#One can be 95% certain, that the range from 22 - 25 CFU/L will contain th
e population mean
```

```
lb.min <- median(Cw.100)-standard_error*qnorm(0.95) #lower bounds 19
ub.min <-median(Cw.100)+standard_error*qnorm(0.95) # upper bounds 21.411
lb.min # #19.3

## [1] 17.84352

ub.min #(23.4) 21.6

## [1] 20.1668
```