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Literature review:
Risks to human health from pathogens in recycled wastewater

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EXECUTIVE SUMMARY

Water shortages and restrictions are becoming increasingly common in New Zealand, so permanent solutions for maintaining adequate supplies of water may be sought in the future. Water conservation should be the first action to address water shortages, but the reuse of treated wastewater is another option. There are also other pressures for the reuse of wastewater, such as sustainable design, promotion by councils or communities and as a substitute for artificial fertilisers. In New Zealand, wastewater discharge to land is currently carried out as a disposal mechanism rather than as a source of irrigation water, but this may still have implications for the health of people living and working nearby.

In 2006, the Australian Natural Resource Management Ministerial Council, Environment Protection and Heritage Council, and the National Health and Medical Research Council, and the World Health Organization (WHO) published guidelines for wastewater reuse. Both used a quantitative microbial risk assessment (QMRA) methodology to assess risks to public health from various reuse scenarios. The Australian Guidelines for Water Recycling (AGWR) are referenced in the international guidelines for health risk assessment and management for non-potable water reuse (ISO 20426:2018) in relation to developing appropriate criteria and mitigation measures where people may be exposed to recycled wastewater, highlighting their ongoing relevance.

The purpose of this review is to highlight recent studies (post-2006) in the literature that can be used by public health officers to prepare advice and inform policies and resource consent applications based on the risk to public health from wastewater reuse, and to identify appropriate mitigation measures for potential adverse health effects. The review focuses on human health issues from pathogenic microorganisms in wastewater for reuse in high income countries because microbial contamination has been identified as the greatest risk associated with wastewater reuse (WHO, 2006).

New information from a review of epidemiological studies is presented. Although most of the studies in this review were related to low- and middle-income countries, the information may be of relevance to New Zealand, as wastewater that is disposed to land may be treated to a lower standard than for wastewater reuse, with potential health risks to residents and workers.

Most of the new literature that is relevant to high-income countries relates to or informs QMRA. Examples from the literature highlight changes to the target pathogens, dose-response models and exposure data since the publication of AGWR. A key area not covered in detail in AGWR was the potential health risk from human exposure to aerosols and dispersed droplets of treated wastewater. These changes and new knowledge may result in changes to the criteria presented in AGWR (e.g. public irrigation, including of golf courses, and use in cooling towers).

The types of wastewater reuse that are most likely to occur in New Zealand are:

- irrigation for public amenities such as sports grounds and parks
- irrigation for agriculture (excluding the production of foods consumed raw)
- reticulated recycle systems used in urban and/or industrial areas.

It is considered highly unlikely that wastewater would be used to irrigate foods that are eaten raw in New Zealand. However, information on the health risks associated with the consumption of foods eaten raw is also included in this review, as spray drift may occur when treated wastewater is disposed to land.

The literature shows that QMRA is a useful tool for assessing the human health risk from the use of recycled wastewater and that local data improve the robustness of the assessment. However, a sensitivity analysis of the inputs is critical, as there is a high level of uncertainty associated with key input data such as the concentration of pathogens in wastewater (raw and treated) and their removal during treatment. Assumptions need to be carefully assessed as some assumptions are not robust. QMRA can assist decision making by enabling the effectiveness of different mitigation measures or interventions to be compared.

1. INTRODUCTION

Water shortages are becoming increasingly common in New Zealand due to factors such as changes in weather patterns, a lack of appropriate infrastructure or poorly maintained infrastructure. Acute water shortages can be managed by water restrictions, but as water shortages become more frequent, more permanent solutions may be sought. Water conservation should be the first action to address water shortages, as this is a more sustainable approach than using water, treating the wastewater stream produced and then reusing the treated wastewater. It also has less potential for adverse effects on human health and the environment. However, the reuse of treated wastewater could be sought as a solution There are also other pressures for reusing wastewater, such as sustainable design, promotion by councils or communities, as seen at Akaroa (CCC, n.d.) or as a substitute for artificial fertilisers due to the presence of nitrogen and phosphorus.

In 2006, the Australian Natural Resource Management Ministerial Council (NRMMC), Environment Protection and Heritage Council (EPHC), the National Health and Medical Research Council (NHMRC) and the World Health Organization (WHO) published guidelines for wastewater reuse (NRMMC et al., 2006; WHO, 2006). Both used a quantitative microbial risk assessment (QMRA) methodology to assess risks to public health from various reuse scenarios, but they had widely divergent water quality criteria and preventive measures for reuse (Leonard et al., 2023). The WHO guidelines (WHO, 2006) focused on low- and middle-income countries that may have issues with food security, a high burden of disease already present in communities and a limited ability to fund high levels of wastewater treatment. By contrast, the Australian Guidelines for Water Recycling (AGWR) had a greater focus on treatment to achieve log reductions of target pathogens but also included preventive measures (NRMMC et al., 2006).

The recent international guideline on health risk assessment and management for non-potable water reuse, ISO 20426:2018, recommends the AGWR methodology for developing risk assessments, appropriate criteria and mitigation measures where people may be exposed to recycled wastewater (ISO, 2018). This highlights the robustness of the work undertaken by the NRMMC, EPHC and NHMRC in 2006.

The purpose of this review is to highlight new studies in the literature that can be used by public health officers to prepare advice and inputs to policies and resource consents based on public health risk from the reuse of wastewater, and to identify appropriate mitigation methods for potential adverse health effects. The review focuses on human health issues from pathogenic microorganisms in reused wastewater for reuse in high income countries because microbial contamination has been identified as the greatest risk associated with wastewater reuse (WHO, 2006). Risks associated with organic chemicals, metals, emerging contaminants and antimicrobial resistance are less frequently considered (Leonard et al., 2023) and are excluded from the current review, as are microplastics.

This review specifically focuses on studies where secondary or tertiary treated wastewater is recycled and assumes that there has been some reduction in pathogen concentrations compared with raw sewage.

A range of studies can provide information about the public health risks associated with wastewater reuse. Epidemiological studies present evidence of links between illness and an exposure pathway and are discussed in section 2. However, it can be difficult to establish a link between a disease outbreak and what caused it. Therefore, risk assessment methodology is used to assess the hazard, pathway, likelihood of exposure and health impact, with the most common approach being QMRA. Examples from recent literature are presented using different input data from the original guidelines. Literature on the inhalation of pathogens from aerosol production is also included, as this pathway was not considered in the WHO or AGWR. The impacts of different mitigation measures which may be assessed through QMRA modelling are also discussed.

QMRA models are highly sensitive to the input data. Since 2006 there have been changes to the choice of target pathogens used in QMRA and more information on the amount of target pathogens in raw wastewater, their removal in wastewater treatment plants and their presence in recycled wastewater infrastructure. There have also been new studies on exposure pathways, particularly for aerosols, and hazard characterisation with new information on dose-response models. An overview of literature is provided on the inputs as the volume of literature in each area can be extensive.

This literature review focuses on the types of reuse that are most likely to occur in New Zealand and includes literature published since the 2006 guidelines were released in relation to:

- irrigation for public amenities such as sports grounds and parks
- irrigation for agriculture
- reticulated recycle systems used in urban and/or industrial areas.

It is considered highly unlikely that wastewater would to be used to irrigate foods that are eaten raw in New Zealand. Social and cultural factors are important for acceptance of the reuse of recycled wastewater (Hajare et al., 2021), and the irrigation of foods consumed raw is likely to be rejected by the general public in New Zealand. Additionally, Māori have established cultural traditions and associated customary practices for managing human waste, particularly in relation to keeping it separate from food (Pauling & Ataria, 2010). However, studies on the reuse of wastewater for growing foods consumed without processing are also included as it is applicable to spray or aerosol drift.

There are three key exposure pathways:

- ingestion
- inhalation
- dermal.

Ingestion and inhalation may cause gastroenteric or respiratory illness in people who live and work nearby, while dermal adsorption may cause skin irritations or wound infections. Helminth infections can also occur through skin contact and can be a substantial human health concern in low- and middle-income countries. However, helminths are not considered a significant human health risk in sewage or sewage sludge in New Zealand (DH, 1992) and the incidence of human helminth infections in New Zealanders is low, so the prevalence of helminth contamination in New Zealand wastewater is also low. Consequently, dermal exposure is not reviewed in detail.

The review also does not cover the discharge of wastewater to groundwater via land, or managed aquifer recharge, or the economic and environmental costs of treating water for reuse.

2. EPIDEMIOLOGICAL STUDIES

2.1 INTRODUCTION

Epidemiological studies may be able to identify associations between an activity and a human illness. However, it is often difficult to find direct links because there may be many sources of illness in a community.

In low- and middle-income countries, wastewater treatment may be of a lower quality than in high-income countries due to a lack of sanitation. There may also be a low level of protection for workers – for example, they may work barefoot and experience high rates of skin diseases and gastroenteritis. By contrast, the most common disease associated with wastewater reuse in high-income countries such as New Zealand is gastroenteritis. This is the focus of this review of epidemiological studies.

Viruses were assessed as presenting the highest risk of gastroenteritis from wastewater reuse in the Australian Guidelines for Water Recycling (AGWR) (NRMMC et al., 2006) and WHO guidelines (WHO, 2006), but gathering data on outbreaks is challenging, as viral gastroenteritis does not have mandatory reporting requirements in most countries. Consequently, it is difficult to estimate the incidence of viral gastroenteritis due to wastewater reuse unless there has been an outbreak or a community has received targeted viral gastroenteritis investigations such as the study on household gastroenteritis in Melbourne by Sinclair et al. (2005). This lack of systematic reporting means there will be substantial under-reporting of gastrointestinal illness. Analysis of acute gastrointestinal illness survey data by Public Health Agency of Canada estimated that 300 cases may go unreported for every reported case (Thomas et al., 2008).

A literature review was undertaken to look for epidemiological studies published since 2006 that identified links between gastroenteritis and the reuse of treated wastewater using the keywords ‘gastroenteritis’, ‘epidemiology’ and ‘wastewater reuse’. The databases searched were Web of Science, ProQuest and PubMed. The articles that were identified as relevant and reviewed in detail covered:

- gastroenteritis in farm workers, households and the community
- gastroenteritis from the consumption of contaminated food.

2.2 FARM WORKERS AND THEIR FAMILIES

Without adequate protection and awareness of the risks associated with using recycled wastewater and poorly treated wastewater, farm workers and their families can have higher rates of gastroenteritis and skin infection. No epidemiological studies since 2006 were identified in the literature sources queried for higher income countries, but a review of 20 epidemiological studies on illnesses from microbial pathogens in farm workers exposed to wastewater in low- and middle-income countries was found (Dickin et al., 2016). Data were collected from stool samples, dermatological examinations and health surveys. The four studies where excreta was used, and two studies with aquaculture are not included in this discussion. Control groups were used in these studies. The review highlighted the prevalence of gastroenteritis and skin problems in the workers and their families in low-income communities in Vietnam, Morocco, South Africa and Eritrea.

Occupation was an exposure pathway in all 14 studies. One study was of the workers only and the rest included their households, with five studies of children only. Risk factors included wastewater irrigation, or living near wastewater irrigated areas, consumption of vegetables, sanitation, hygiene, and water source. Analysis of stool samples identified helminth infections were most frequently present (six studies). Gastrointestinal illness from *Giardia* or unspecified protozoa were identified in five studies and *Salmonella* in two studies. Unspecified sources of gastrointestinal illness were reported in four studies. Multiple gastrointestinal pathogens were reported in four studies. Two studies identified skin problems; one reported gastrointestinal illness as well.

In a meta-analysis of epidemiological studies on the health of farm workers mostly from low- and middle-income countries, which included studies prior to 2006, Adegoke et al. (2018) found that exposure to wastewater was associated with poor health. The odds ratio in this analysis was greater than 1 (1.65; 95% confidence interval: 1.31, 2.06), indicating that exposure was associated with a higher risk of illness than non-exposure. Soil was also identified as a potential pathogen exposure pathway for farm workers.

2.3 COMMUNITY

Links between poor health outcomes and proximity to wastewater 'reuse' in Thailand were reported by Ferrer et al. (2012). In this case, 'reuse' occurred as the canals used by the communities were contaminated with sewage. Such contamination of waterways is unlikely in New Zealand, but disposal by spray irrigation of wastewater with low levels of treatment is a potential exposure route.

Where spray irrigation is used, aerosolised pathogens can travel further than the wetted area and be inhaled by workers or local community. Droplets and aerosols may also settle on food that is later consumed or on surfaces where hand-to-mouth ingestion may occur. However, no recent cases of gastroenteritis related to the reuse of wastewater were found in the literature review. This could be due to the high standards of wastewater treatment in guidelines for high income countries e.g. AGWR (NRMMC et al., 2006), or a lack of reporting (Thomas et al. 2008).

Legionella is an opportunistic pathogen that is often present in drinking water. It can persist and accumulate in wastewater and water reticulation systems and has the potential to be aerosolised. None of the epidemiological studies reviewed linked outbreaks of Legionellosis with recycled wastewater. However, *Legionella* are present in wastewater and therefore a potential pathogen (Caicedo et al., 2019; Olsen et al., 2010).

2.4 CONSUMERS OF CONTAMINATED FOOD

Recycled wastewater may be used to irrigate private food supplies or commercial food crops. No published studies of gastroenteritis outbreaks that could be directly attributed to wastewater reuse and food irrigation were found for high-income countries. This is likely due to the very high wastewater treatment criteria and the guidelines for the use of wastewater for irrigation of food crops in high-income countries, which may mitigate risk from wastewater reuse compared with other sources of infection in the community. However, Benjamin et al. (2013) identified that there could be a number of critical control points in food processing, preparation and handling at which contamination may occur, in addition to irrigation with recycled wastewater.

In a review of epidemiological studies, Dickin et al. (2016) showed that four out of the 14 studies mentioned in section 2.3, identified consumption of vegetables as a risk factor, but also noted water, sanitation and hygiene as other risk factors. *Giardia* was the most commonly identified pathogen found in stool samples (3/4 studies), and general gastroenteritis in 2/4 studies. Helminth infection was also reported in 2/4 studies. All four studies were in low- and middle-income countries.

3. QUANTITATIVE MICROBIAL RISK ASSESSMENT (QMRA)

In the absence of robust epidemiological data, QMRA is a useful approach for assessing health risk. Other approaches also exist that may be more applicable to local conditions – for example, Beaudequin et al. (2015) proposed a systems model that could account for the high amount of variability in characterising each of the four inputs into a QMRA, while Troldborg et al. (2017) used fuzzy logic. However, QMRA is the most commonly used model and is recommended by ISO (2018) for assessing risks associated with wastewater recycling where there is the potential for humans to be affected. The four key components of QMRA are shown in Figure 1.

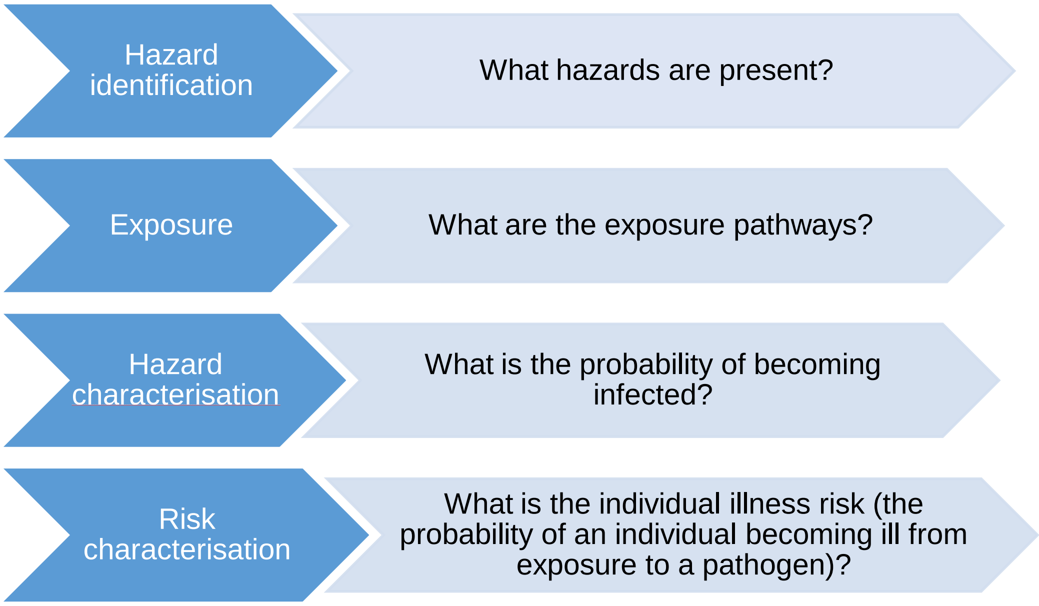

Figure 1. Key stages of QMRA

In 1999, Haas et al (1999) published a book detailing QMRA methodology, (updated Haas et al., 2014). McBride et al. (2013) provides a useful summary of QMRA, highlighting issues around the components of the model. While target pathogens and exposure pathways can be identified, there is more uncertainty around input variables such as the:

- concentrations of target pathogens in sewage
- geographical, seasonal and between-year variability in concentrations of pathogens
- methods of calculating the concentrations of pathogens in raw sewage
- log pathogen reduction in the wastewater treatment train
- log pathogen reduction from preventive barriers
- dose-response model and application to vulnerable communities
- volume ingested

- exposure duration.

While inputs may be highly variable or associated with a lot of uncertainty, a strength of the methodology is the ability to compare the impact of interventions or mitigation measures by varying one input and keeping the other inputs constant. When undertaking a QMRA, it is necessary to include an assessment of how sensitive the results are to changes in the input data, particularly where assumptions have been made. Sensitivity analyses are discussed in relation to individual reuse activities in the following sections.

QMRA models assess risk in different ways. Some models can be used to estimate the probability of infection from a single exposure, as used by Mori & Smith (2023) to predict the risk of *Legionella* infection for farm workers who were exposed to irrigation water. Other models include the frequency of exposures over a time period – for example, the risk of *Legionella* infection and illness for someone working outside near a cooling tower (Hamilton et al., 2018). Most QMRA models only include primary infection, but secondary infection rates may be important to consider. Barker et al. (2013) used a secondary infection rate of 14-18% to model the risk of illness for a range of pathogens for a small, confined community in Antarctica.

To contextualise the output of a QMRA (probability of infection/illness), the output probability may be compared to a health target. Health targets are commonly expressed in two ways in QMRAs:

- Disability-adjusted life years (DALYs), which estimate the burden of disease for a country or region. These estimates can then be divided by the number of cases to give DALYs/case or by the number of people in the population to give DALYs/person. Estimates are usually based on incident cases in a given year, although the burden may extend over a number of years. The DALY estimate is made up of two components: years of life lost, a measure of the burden due to mortality; and years of life lived with disability, a measure of the burden due to morbidity. Health impacts are weighted in terms of severity (from a minor inconvenience through to death), which is multiplied by the duration of the effect. This can be modelled based on single or multiple exposures. The DALY health target is normally 10-6 pppy, based on WHO (2006).

- Annual risk of infection, which does not take account of the severity of the disease and may also not be equal to illness for pathogens where not all people who are infected become ill.

- Risk of infection or illness from an exposure event.

The reference health-based targets proposed for the reuse of wastewater in WHO guidelines (WHO, 2006) and AGWR (NRMMC et al., 2006) are $<10^{-6}$ DALYs per person per year (pppy). An annual risk of infection of $<10^{-4}$ pppy, may also be used as a health target, based on the United States Environmental Protection Agency drinking water regulations (EPA/USDA-FSIS, 2012). There are important differences between the guidelines provided in WHO (2006) and AGWR (NRMMC et al., 2006), however. The WHO guidelines (WHO, 2006) were derived for communities with high levels of disease and limited funds to meet wastewater treatment and sanitation requirements, as well as other beneficial infrastructure such as safe drinking water. The WHO guidelines (WHO, 2006) also assumed that farm workers could make an informed choice regarding working conditions that managed the health risk and that behavioural preventive measures, such as washing food and withholding periods between harvesting food and consumption, would be effective, as the level of wastewater treatment would be low due to a lack of funds. By contrast, AGWR (NRMMC et al., 2006) relied more heavily on wastewater treatment and considered withholding periods and washing food to be weak preventive measures, that were not used in the risk assessment.

Lower targets have been proposed than are presented in WHO (2006). Mara et al. (2009) proposed an increased DALY of <10^{-4} pppy for farm workers and <10^{-5} pppy for consumers of products for low- and middle-income countries due to the existing disease burden in such communities. Mok et al. (2014) also proposed using the higher DALY target of <10^{-4} pppy based on the local incidence of illness. An even lower health target of 10^{-2} pppy for the annual risk of infection was proposed by Seiss et al. (2022) based on survey data that showed 9 out of 10 adults in Germany experience a case of gastroenteritis per year. This gastroenteritis rate is within the range reported from the Melbourne survey undertaken by Sinclair et al. (2005), which gave a rate of 0.7–1.06 cases pppy, with norovirus being identified in 10.7% of cases. These surveys also indicated that a number of potential sources are associated with gastroenteritis within a community.

Advances have been made in the science underpinning QMRA since the methodology was applied in the AGWR (NRMMH et al. 2006) and WHO guidelines (WHO, 2006). This review uses a broad-brush approach to inform the scope and/or assessment of QMRA. It is not intended to provide detailed data for undertaking a QMRA, as this requires highly specialised knowledge with regard to both the choice of input data and understanding differences in methodologies. Additionally, air modelling expertise is required to assess the risk of spray drift and aerosols.

3.1 TARGET PATHOGENS

Pathogens in wastewater will reflect the incidence of illnesses such as gastroenteritis or respiratory infections in the community, but not all pathogens will be present at all times, particularly in small communities (Hewitt et al., 2011). Therefore, the AGWR (NRMMC et al., 2006) and WHO guidelines (WHO, 2006) identified the following target pathogens on for the QMRA: *Campylobacter* (bacteria), rotavirus (virus) and *Cryptosporidium* (protozoa).

Viruses were considered the pathogens that presented the highest risk of illness due to their environmental resilience and the low infectious dose for viruses such as norovirus (Teunis et al., 2008a), although *Campylobacter* has a higher DALY value than norovirus in New Zealand (Cressey et al., 2013) and it has been reported that *Salmonella* and *Campylobacter* both have higher DALY values than norovirus in Australia (Gibney et al., 2014). Olivieri et al. (2014) chose different target pathogens and different dose-response models in their QMRA of California's water reuse for food production. The rotavirus dose response model was selected as the dose-response model for norovirus (Teunis et al., 2008a) and was assessed as being less conservative. However, it is noted that they based the concentration of the rotavirus on enterovirus concentrations. They also used *Escherichia coli* O157 as the bacterial target pathogen, despite noting that data on *E. coli* O157 was very limited.

The literature highlights that QMRA is highly sensitive to the concentration of pathogens in wastewater (raw and treated). Changing the target pathogen may change the log pathogen reduction required in a wastewater treatment plant (WWTP), so recent studies on various target pathogens are reviewed in this section.

3.1.1 Pathogens in raw wastewater

Rotavirus was used as the target pathogen in the Australian and WHO guidelines due to the absence of a suitable dose-response model for norovirus at the time (NRMCC et al., 2006; WHO, 2006). Consequently, the log pathogen reductions required for wastewater treatment for different water reuses in AGWR (NRMMC et al., 2006) are based on rotavirus. However, there were insufficient data available on the concentration of rotavirus in raw sewage in Australia at the time AGWR was produced, so the prevalence of adenovirus[1] in wastewater was used instead, based on the similarity between adenovirus, rotavirus and norovirus concentrations when measured using the polymerase chain reaction (PCR). Since then, a dose-response model developed by Teunis et al. (2008a) showed that norovirus could be infectious at very low doses, so norovirus is now used. It should also be noted that the incidence of rotavirus infections in New Zealand has substantially decreased following the development of a vaccine for this virus.

The use of rotavirus as a target pathogen had some advantages over norovirus, as it can be cultured which indicates it is viable and therefore likely to be infectious. By contrast, quantitative PCR (qPCR) may also detect pieces of genetic material from dead and degraded microorganisms, resulting in an overestimate of risk. Mok & Hamilton (2014) used a factor of 1000 to harmonise concentrations of rotavirus determined by culture with concentrations determined by qPCR, based on Rutjes et al. (2009). The infectivity of enterovirus can also be assessed by culture, and this virus has been used in other QMRAs (Seto et al., 2018). The infectivity of norovirus has been difficult to establish due to difficulty in culturing it, and this lack of knowledge about infectivity has been identified as an area of uncertainty in norovirus QMRAs. However, Estes et al. (2019) reported the growth of norovirus and proposed a factor of 100 to estimate infectious norovirus, although Simhon et al. (2020) highlighted that criteria should not be changed until norovirus culture becomes routinely established. The analytical methods used by different researchers also need to be taken into account, as the result may differ depending on the method used. For example, Teunis et al. (2008a) used a primer with one target, while Simhon et al. (2020) used primers that had two targets in norovirus, resulting in higher concentrations being observed.

Since 2006, more data on norovirus concentrations in raw sewage have been published. These data show that concentrations vary by country, depending on the level of disease in the community, the volume of wastewater produced per person and by season. Seis et al. (2020) reported data on norovirus genogroup II (NoV GII) from 44 European WWTPs and found that the mean ranged from 4.1 to 7.6 $\log_{10}$ genome copies (GC)/L. However, there was also variability in the data within individual WWTPs, with one WWTP exhibiting 6 $\log_{10}$ variation between individual samples. Meta-analysis of norovirus concentrations in raw wastewater, obtained by a systematic literature review highlighted seasonal and geographical differences (Eftim et al. (2017). Norovirus was more prevalent in winter and spring than in summer and autumn, and the annual mean norovirus concentration was 4.0 $\log_{10}$ GC/L for North America, 5.1 $\log_{10}$ GC/L for Europe, 6.4 $\log_{10}$ GC/L for Asia and 2.4 $\log_{10}$ for New Zealand (from the study by Hewitt et al., 2011). By contrast, a later study over a one–year period by Gyawali et al. (2021) reported median norovirus concentrations from four New Zealand WWTPs of 4.96 ± 0.64 $\log_{10}$ GC /L norovirus genogroup I (NoV GI) and 6.25 ± 0.66 $\log_{10}$ GC /L NoV GII. Seis et al. (2022) also reported significant changes in the concentration of norovirus, with >2 $\log_{10}$ variation over 7 weeks (4.3-7.0 $\log_{10}$ GC/L) in samples collected between October and December. As this was not during the peak incidence of norovirus (winter, January–February) it would be expected that peak sewage concentrations would be higher. These studies highlighted the importance of local pathogen data and seasonal variation.

Norovirus infection is not a notifiable disease in most countries, so data on incidence is limited. However, there are studies from Japan and Germany which data have demonstrated

[1] Although most adenoviruses cause respiratory disease.

that the concentration of NoV GII in raw sewage can be related to the incidence of gastroenteritis. In Japan, Kazama et al. (2017) showed that there was a cross-correlation between gastroenteritis cases and the concentration of NoV GII but not NoV GI in sewage. In Braunschweig, Germany, Seis et al. (2022) also found a strong correlation between reported cases of NoV GII and the concentration in the raw sewage. Seis et al. (2022) used the observed correlation to calculate the concentrations of norovirus for 17 years of epidemiological data. The results also showed variation in the incidence of norovirus and highlighted that there could be significant variability in sewage concentrations of norovirus between years as well as within a year. Therefore, the long-term monitoring of wastewater was recommended to address this variability.

Faecal shedding rates were also used to estimate the concentration of norovirus in raw sewage. Mok et al. (2014) and Barker (2014) used shedding data from the same small study of 16 people undertaken by Atmar et al. (2008). Using different studies for the incidence of norovirus in the community, different concentrations of norovirus in sewage weree derived. Mok et al. (2014) used estimates from a 2004, four week, nationwide household survey of gastroenteritis across Australia, while Barker (2014) used data reported by Sinclair et al. (2005) from a 1997-1999 randomised control trial in Melbourne on the incidence of gastroenteritis and norovirus infection. The mean concentrations of norovirus calculated in these studies were 7.8 $\log_{10}$ GC /L by Mok et al. (2014) and 9.0 $\log_{10}$ GC/L (range: 0-10^{10}/L) by Barker (2014). Barker concluded that shedding rates resulted in overestimation of norovirus concentrations compared with published data on concentrations in raw sewage, even allowing for different recovery rates. Barker (2014) proposed possible reasons for the differences between measured and predicted norovirus concentrations, including differences in the norovirus incidence across different countries and the lower amount of wastewater per person in Australia due to water restrictions. Norovirus as a percentage of reported cases of gastroenteritis varied by an order of magnitude across countries, with a value of 0.013 reported in England and 0.113 reported in Australia (Barker 2014). Barker (2014) reported results for three QMRA based on faecal shedding rates, norovirus concentrations measured in Japan and norovirus concentrations measured in Japan multiplied by a factor of 10. Sensitivity analysis showed that the concentration of norovirus accounted for much of the variability. While health targets were met using Japanese data and Japanese data multiplied by 10, the targets were not met using faecal shedding rates.

Target bacterial pathogens also vary. QMRA have used pathogenic *E. coli* (Chhipi-Shrestha et al. 2017; Olivieri et al 2014) and *Salmonella* (Chhipi-Shrestha et al. 2017). Recent studies have also looked at opportunistic pathogens such as *Aeromonas* may also present a risk to immunocompromised people and cause gastroenteritis in healthy people. *Mycobacterium* may also be present and could be a potential risk through inhalation of aerosols or from contaminated soil infecting open wounds. *Legionella* infection also occurs through inhalation, not the oral–faecal exposure pathway, and *Legionella* can be present in potable water systems and, due to their ecology, can persist through wastewater treatment systems and therefore accumulate in recycled wastewater infrastructure in biofilms and through ingestion by amoeba. The risk of *Legionella* infection through inhalation is discussed in sections 4.2.1, 5.1, 5.3, 5.4 and 6.

Recent literature presents a wide range of target pathogens that may present a health risk to different members of the community, such as children or those who are immunocompromised. Haas et al. (2014) identified that up to 40% of the population may be sensitive (pregnant women, the elderly, infants and the immunocompromised), so site-specific risk assessments may need to consider the risk to local sensitive populations.

3.1.2 Pathogens in treated wastewater

Wastewater may be treated to remove pathogens in a variety of ways. For recycle uses that present a high risk to human health, disinfection and further treatment such as reverse osmosis may be added to the treatment train. A 'log pathogen reduction' is the common metric that indicates the level of pathogen removal required, i.e. the difference between the concentration in the influent and effluent. Log pathogen reduction is additive for the steps along the wastewater treatment train and may be determined by changes in the concentration of specific pathogens or surrogate microbial indicators. AGWR (NRMMC et al., 2006) provides a table of the range of log pathogen reductions for different types of pathogen classes after wastewater treatment.

While literature-based log pathogen reductions, such as those given in AGWR (NRMMC et al., 2006), may be used in some QMRA studies, pathogen reduction at local WWTPs is determined in others (Bailey et al., 2018; Mok et al., 2014; Seto et al., 2018; Simhon et al., 2020). Local data also allows the dominant pathogens to be identified and assessed for risk. Bailey et al. (2018) reported the health target was met for recyulced water iirgation for adenovirus was met, but not always for *Salmonella*, *Cryptosporidium* and *Giardia*. more prevalent in recycled wastewater than NoV II (Bailey & Sobsey 2022). Log pathogen reduction values can be widely different, even for the same type of WWTP, particularly for waste stabilisation ponds (WSPs) (Mok et al., 2014). Site-specific studies have shown that the actual reduction at a WWTP may be less than is required by regulatory authorities or specified in guidelines such as AGWR (NRMMC et al., 2006). For example, Bailey et al. (2018) found that the reduction of adenovirus was only 1.9 $\log_{10}$, which is much less than the 5 $\log_{10}$ reduction required by regulation. This study also highlighted the difference between using integrated cell culture PCR (ICC-PCR) and qPCR to calculate log pathogen reduction. An adenovirus log reduction of 2.8 was calculated by ICC-PCR and 1.9 by qPCR (Bailey et al., 2018).

There are many studies on pathogen removal, so this section provides an overview on the following specific aspects:

- indicators of pathogen removal
- metagenomics, a genetic technique that can be applied to increase our understanding of pathogens that are not routinely analysed
- WSPs, which are a common form of treatment in New Zealand and have highly variable log pathogen reduction values (NRMMC et al., 2006).

Traditionally, *E. coli* or faecal coliforms are the faecal indicator bacteria (FIB) that are used as criteria to indicate the removal of pathogens from wastewater. However, studies that have measured FIB and pathogens in treated wastewater have highlighted the poor correlation between concentrations of FIB and pathogenic bacteria and viruses in recycled wastewater (Ajibode et al., 2013; Bailey et al., 2018; Benjamin et al., 2013; Moazeni et al., 2017; Simhon et al., 2020; Verbyla and Mihelcic, 2015). Field studies by Benjamin et al. (2013) also highlighted the poor correlation between concentrations of pathogens (*Salmonella* and toxic *E. coli*) and indicator *E. coli*, but noted that other sources of pathogens may be present in a farming environment. It has also been reported that opportunistic pathogens such as *Aeromonas*, *Legionella, Mycobacterium* and *Pseudomonas* occur more frequently in wastewater than the indicator bacteria (Jjemba et al., 2010).

Verification of WWTP performance is used to confirm adequate removal of bacteria, viruses and protozoa has occurred. AGWR (NRMMC et al., 2006) details verification processes, the frequency of verification and the target pathogens to use (e.g. adenovirus and *Cryptosporidium*), or surrogates which may be used for viruses (e.g. MS2 coliphage) and protozoa (e.g. *Clostridium perfringens* spores). In North Carolina, only surrogates are used to assess pathogen removal, and Bailey et al. (2018) highlighted the difficulty of demonstrating that the required log pathogen reduction has occurred when the concentrations of the target surrogate in raw wastewater were below the log reduction target. For example, a 5 log_{10} removal is required for viruses, but the concentrations of coliphage, the target surrogate were only 4 log_{10}/L in raw wastewater.

Matching the behaviour of a surrogate to the target pathogen is critical to accurately determine log pathogen reduction. Bailey et al. (2018) showed that the surrogates proposed for viruses (somatic coliphage and F-RNA phage) and protozoa (*Clostridium perfringens* spores) could be more readily removed than the target pathogens (*Cryptosporidium, Giardia* and adenovirus). As mentioned above, the analytical method e.g. culture or qPCR is also important with higher removal by ICC-PCR compared to qPCR for adenovirus (Bailey et al., 2018).

The surrogate also needs to have the same susceptibility to the treatment as the pathogen. MS2 coliphage is commonly specified as the surrogate for virus removal by ultraviolet (UV) light. However, UV radiation from sunlight is rapidly attenuated in the water column in a WSP, thus the main virus removal mechanism is indirect inactivation. While MS2 coliphage is a good surrogate for virus removal where direct UV inactivation occurs, it is less resistant to indirect sunlight inactivation than common viruses such as adenovirus and rotavirus (Verbyla & Mihelcic, 2015).

Metagenomics provides a description of the communities of microorganisms in a particular medium and can provide information on the prevalence of pathogens that are not commonly analysed. In an analysis of bacterial communities in 37 samples from four regionally separated WWTPs in the USA, Kulkarni et al. (2018) found that while the abundance of bacterial genera in the influent to the four WWTPs was not significantly different, the composition of the microbial communities in the effluent did differ. The opportunistic pathogen *Legionella* was in the top 10 most abundant species in the effluent at two of the four WWTPs and had a higher prevalence in the effluent than the influent. Similarly, *Mycobacterium* was in the top 10 most abundant species in the effluent at three of the WWTPs, with the same or higher levels of abundance in the effluent compared with the influent.

WSPs are common WWTPs in New Zealand and consist of a series of ponds that hold wastewater for a specific length of time (hydraulic retention time). Pathogen removal can occur through mechanisms such as sedimentation, UV radiation from sunlight and die-off. The hydraulic retention time is a key operating parameter but, in practice, the actual hydraulic retention time may differ from the theoretical hydraulic retention time due to factors such as hydraulic short circuiting. A review of field studies on the removal of viruses from 71 WSPs showed a weak correlation between virus removal and the theoretical hydraulic retention time (Verbyla & Mihelcic, 2015). On average, 1 log_{10} reduction of viruses was achieved with hydraulic retention times between 14.5 and 20.9 days, and the 95th percentile[2] virus removal rate was 54 days/log_{10} reduction (Verbyla & Mihelcic, 2015). Mok et al. (2014) highlighted the inconsistency in the log pathogen reduction from WSP treatment and recommended that disinfection processes be used after WSP treatment to ensure that log reduction targets could be met routinely. The New Zealand Ministry for the Environment

[2] 95th percentile is the value which is greater than 95% of the data in a dataset

was cited, reporting that mechanical UV treatment was the most commonly used disinfection treatment for WSP effluent in New Zealand, having replaced chlorination due to concerns about the potential formation of toxic byproducts with this method. However, Verbyla and Mihelcic (2015) indicated that there is very little information on the effectiveness of artificial UV on WSP effluent. Additionally, no studies were found on the effectiveness of peracetic acid or ozone on virus removal after WSP treatment (Verbyla & Mihelcic, 2015).

In a New Zealand study, two studies on concentrations of noroviruses in WWTP influent and effluent have been published. The details of the studies and results for norovirus are summarised in Table 1.

Table 1 Summary of data on norovirus in WWTP influent and effluent in New Zealand

Type of norovirus	Influent concentrations $\log_{10}$ GC/L	Effluent concentrations $\log_{10}$ GC/L	No. WWTP	No. samples/ WWTP	Period	Reference
NoV GI	2.1-4.6*	2.2-5.1*	10	3	November-April	Hewitt et al., 2011
NoV GII	2.2-5.5*	2.9-5.5*				
NoV	5.0-7.0	3.0-5.0	4	4	1 year	Gyawali et al. 2021

*not all samples were positive for norovirus

In Hewitt et al. (2011) when norovirus was present, the concentrations in the influent and effluent were in a similar range indicating that low levels of removal may occur in WWTPs. This study also showed that WWTP size was not related to concentrations of norovirus in raw sewage. Additionally, higher variability in concentration was observed in small WWTPs (<4000 people) compared with medium and large WWTPs. This is because as the population size increases, the likelihood that some portion of the population will be excreting viruses also increases, thus increasing the presence of viruses in wastewater. In the second study Gyawali et al. (2021) reported higher mean concentrations of NoV GI and GII, and removal of norovirus in this study was more evident.

3.1.3 Pathogens in recycled wastewater infrastructure

Treated wastewater will still contain some organic matter and potentially pathogens, giving it a higher potential for microbial regrowth or the accumulation of pathogens compared with drinking water. This presents a potential human health risk when water is reused.

Low levels of residual chlorine (Li et al., 2013) and the presence of organic matter (Jjemba et al., 2010) have been identified as factors that support pathogen regrowth. The literature has shown that faecal pathogens such as *Salmonella* have the potential for regrowth, which could be a health risk where recycled water is stored for long periods before reuse (Li et al., 2013). Opportunistic pathogens also present a potential risk.

It has been reported that *Legionella*, *Aeromonas* and *Mycobacterium* have higher concentrations in treated wastewater than in raw influent in recycled wastewater infrastructure (Ajibode et al., 2013; Jjemba et al., 2010). *Legionella* and *Aeromonas* were found in 40% of samples and *Mycobacterium* were present in 30% of samples of treated, for disinfected (UV mechanical treatment or chlorination) recycled wastewater at two WWTPs over a 15-month period (Ajibode et al., 2013). Additionally, amoebic activity, which can be protective for *Legionella*, was detected in approximately one-third of the samples. Similarly, a statistically significant increase in concentrations of *Legionella* and *Mycobacterium* was reported in the effluent from four WWTPs in different US states in a 12-month study (Jjemba et al., 2010).

Regrowth of *Legionella*, *Mycobacterium* and *Aeromonas* within the recycled wastewater distribution system occurred at different rates for the different pathogens and treatments (Ajibode et al., 2013). From the point of chlorination to 7.2 miles away, *Legionella* increased from approximately 1.3 log_{10} colony-forming units (CFU)/100 mL to approximately 3.2 log_{10} CFU/100 mL, while *Aeromonas* increased from 1.3 log_{10} CFU/100 mL to 2.5 log_{10} CFU/100 mL. By contrast, *Mycobacterium* concentrations were lower and more constant along the distribution system at approximately 1-1.5 log_{10} CFU/100 mL. From the point of UV treatment to 7.6 miles away, *Legionella* concentrations decreased (approximately 2.1 log_{10} CFU/100 mL) but *Mycobacteria* increased from 1 log_{10} CFU/100 mL to 2 log_{10} /100 mL and *Aeromonas* increased from 1.5 log_{10} CFU/100 mL to 3.5 log_{10} CFU/100 mL. A chlorination boost at 11.7 miles had minimal effect on regrowth along the 24-mile-long distribution system.

Legionella and *Mycobacterium* were also reported at different stages of an irrigation treatment train, with an increase in abundance being observed between mechanical UV treatment, an open-air pond where storage occurred and the pumphouse inlet for spray irrigation for five out of six samples (Kulkarni et al., 2018). *Legionella pneumophilia* was detected after UV treatment but not at the inlet to the pumphouse, while *Legionella feeleii* was detected at the pumphouse. *Legionella feeleii* is ubiquitous and in rare cases can cause pneumonia in immunocompromised people (Lee et al., 2009; Vaccaro et al., 2021). However, most tests are specific for *L. pneumophila* serogroup 1, so the role of other serotypes such as *L. feeleii* in respiratory infections may be underestimated (Vaccaro et al., 2021).

Johnson et al. (2018) also reported the presence of *Legionella,* determined by culture, in 11 out of 19 recycled wastewater utilities, with 15 out of 38 samples testing positive – five from the point of compliance and 10 from the distribution system. Six utilities were analysed in more detail, with samples for *Legionella* being collected from the treatment plant effluent, at the storage facility and within the distribution system (115 samples). *Legionella* was detected in the effluent at five out of six WWTPs and generally increased in concentration along the recycled wastewater infrastructure chain. Factors associated with higher concentrations of *Legionella* included free chlorine residuals of <0.2 mg/L, low nutrient removal in the WWTP and closed storage tanks. However, chloramine appeared to be an effective disinfectant. Temperatures in New Zealand will be lower than in this study (the lowest temperature was 23°C), but *Legionella* may present a potential risk at certain times of the year as it can grow at 20-40°C.

3.2 EXPOSURE AND HAZARD CHARACTERISATION

People may be affected directly or indirectly by wastewater reuse. AGWR (NRMMC et al., 2006) considered exposure from municipal and private garden irrigation, the consumption of food from irrigated crops, toilet flushing, washing machine use, firefighting, and cross-connections in water networks where drinking water may be accidentally connected to sewage or stormwater. A key area where there has been more research since AGWR was published, is the inhalation or ingestion of pathogens in aerosols.

When assessing or scoping a QMRA, it is important to check that the pathways and data used for exposure reflect local conditions. Input data for QMRAs are often obtained from a small number of studies or a general value is applied (e.g. specified buffer distances from spray irrigation). Assumptions are also made to allow complex environmental conditions to be modelled (e.g. constant air stability conditions or wind speed, the shape of the terrain). Therefore, it is important to assess if the assumptions are appropriate and reflect the local conditions. It is also important to consider the presence of any vulnerable people who may be affected in a specific location, such as pre-school- or school-aged children or immunocompromised people.

3.2.1 Ingestion

People can ingest pathogens directly or indirectly. Direct ingestion may occur from the consumption of pathogens in water, food or soil, while indirect ingestion may occur from contact with surfaces where recycled wastewater has deposited (e.g. children touching playground equipment contaminated by spray irrigation and then putting their hands in their mouths). Most studies found in this review referenced the same studies or papers that were used in the QMRA modelling presented in AGWR (e.g. Troldborg et al., 2017). However, Simhon et al. (2020) used a median volume of 6 mL for ingestion during golfing, based on data used by Olivieri et al. (2007), which is greater than the 1 mL ingestion volume used in AGWR (NRMMC et al., 2006), so the results may differ.

3.2.2 Aerosols – indirect ingestion and inhalation

Spray drift and aerosols are exposure routes that were not assessed in detail in AGWR (NRMMC et al., 2006). While an ingestion rate was given for pathogens that are inhaled into the pharynx and then swallowed, a buffer zone for spray irrigation was specified, and other mitigation measures that minimise spray drift and aerosol generation were provided. The deposition of particles containing pathogens onto the lungs was not assessed due to insufficient data being available. However, more recent studies have considered inhalation and ingestion from exposure to airborne particles in more detail.

As well as the ingestion of contaminants that have landed on surfaces or food as droplets, the inhalation of aerosols containing pathogens such as *Legionella* or adenovirus may cause respiratory disease. Wastewater reuses that generate aerosols include spray irrigation, toilet flushing and cooling towers. The international standard ISO 20426:2018 (ISO, 2018) and the European regulation EU 2020/741 (EU, 2020) require the potential impact on health from aerosols to be assessed and provide water quality criteria for *Legionella*. In New Zealand, spray irrigation of wastewater occurs as a disposal method rather than for irrigation, but the impact of aerosols and spray drift are also important to assess for this type of application.

Particles with an aerodynamic diameter of more than 50 µm are likely to rapidly deposit near the site where they are generated, but smaller particles may travel substantial distances away from the source and present a hazard to workers or residents. Pathogens in these aerosols may deposit in the pharynx and be swallowed or, if smaller than 10 µm, may deposit on the lungs. Due to the difficulties in modelling aerosol inhalation, AGWR (NRMMC et al., 2006) focused on mitigating spray drift and aerosol production by specifying irrigation with low risers and large droplet size.

While the wetted area left by spray irrigation can be easily identified, it may not represent the extent of dispersion of larger droplets. Molle et al. (2016) showed that pathogens may also deposit on surfaces away from the wetted area and therefore potentially be ingested. Field data showed that water sprayed at a rate of 4 mL/hr/m^2 would be deposited beyond the wetted area at a distance of 50 m from a sprinkler head set at 0.5 m above ground (i.e. 40 mL/m^2 in 10 hours) at a wind speed of 5 m/s. Thus, with daily deposition and slow die-off times, pathogens could accumulate on surfaces.

Pathogens on bare soil may also be aerosolised. Girardin et al. (2016) reported that 11-15% of viruses applied to soil in a wind tunnel at wind speeds of 3-7.8 m/s were aerosolised – 89% within 30 minutes and the remainder up to 10.6 days after deposition.

Aerosols can be inhaled into the lungs as well as swallowed from deposition in the nasal passage during irrigation. The amount of pathogen inhaled can be determines using air dispersion models and concentrations of aerosolised pathogens. Air dispersion modelling is complex due to the variable nature of wind (it is rarely a constant speed), the three-dimensional (3D) nature of dispersion, air stability conditions, the geometry of the local environment and pathogen die-off in the air. There are a number of air dispersion models that can be applied but, because of this complexity, air dispersion modelling requires highly specialist practitioners.

Some models assume that 100% of aerosolised pathogens are inhaled, while others use data from air samplers to provide field data on the concentration of pathogens in the air that may be inhaled. An air sampler can ‘inhale’ the contaminated air at a given flow rate to match the breathing function of human lungs. Viruses are difficult to capture by samplers due to their small size (0.030-1 µm). Verreault et al. (2008) highlighted difficulties in obtaining good virus field data using impinger air samplers, which trap microorganisms in a liquid. Airborne viruses can also be measured by sedimentation onto filters with different surface properties in open Petri dishes. Courault et al. (2017) found that the impinger and Petri dish methods gave comparable results, but this was dependent on the filter used in the Petri dish. An impact air sampler can differentiate by particle size to determine the respirable volume more accurately. However, as discussed in Verreault et al. (2008), all sampling methods have large variability in terms of capture.

Courault et al. (2017) determined the concentrations of viral pathogens in wastewater and above the WWTP spray irrigated area using impinger air samplers and sedimentation. They found that rotavirus, NoV GI, enterovirus, hepatitis E virus and hepatitis A virus were detected in low concentrations in the air above the WWTP train and/or above the crops on at least one occasion, with hepatitis E the most prevalent virus. These data were used in a QMRA model discussed in section 4.2.1.

Exposure at a given distance from the point of application can increase due to increases in wind speed. Using a wind tunnel, fluorescent dye tracers and conditions of 90% humidity to avoid the effects of evaporation, Cornacchia et al. (2020) demonstrated that at higher wind speeds the air plume was more concentrated and more compact when measured at 12 m. Impinger samplers captured 2.93% of the sprayed volume, but as wind speed increased the droplet size distribution shifted towards smaller sized droplets and the deposited volume decreased with increasing wind speeds greater than 2 m/s.

The evaporation of aerosols with distance will also affect exposure. Tomas et al. (2019) used field data from short-term studies to improve modelling of aerosol evaporation during transport from an irrigator and found that a combination of key evaporation demand variables and Weber or Reynolds numbers gave a better fit than previous empirical models.

Models of the die-off of pathogens vary, and very little has been published on the die-off of pathogens as aerosols. No die-off is considered for inhalation near the source, such as toilet flushing, but a variety of models have been used for spray irrigation, as discussed in sections 4 and 5. The model of Simhon et al. (2020) avoided including die-off, as the parameters were set by assuming that golfing occurs first thing in the morning to reduce the impacts of temperature, sunlight and desiccation.

Specific examples of QMRAs for the inhalation/ingestion of aerosols are discussed in the relevant sections below.

3.2.3 Frequency of exposure

It is important to assess if the input values used in a QMRA reflect local conditions and habits and should be noted that some assumptions may not reflect New Zealand conditions. Local weather conditions will determine the frequency of irrigation, and work practices will affect occupational exposure. For example, one QMRA model for occupational exposure to *Legionella* had people working 265 days/year (Hamilton et al., 2018), while another study had workers exposed 365 days/year (Courault et al., 2017). These models will have overestimated exposure as they did not account for statutory holidays, annual leave provisions in New Zealand or weekends.

Studies have also shown that there is high variability in food consumption between countries. For example, Mok & Hamilton (2014) reported high variability in the frequency and amount of lettuce consumed per person between Asia and Australia.

3.2.3 Dose-response models

A dose-response model estimates the probability of a person becoming infected given a specific quantity of pathogens. Whether a person becomes infected may depend on many things, including their age, state of health and natural resilience. In addition, not everyone who is infected becomes ill because immunity can be acquired through a previous infection. Teunis et al. (2008a) proposed that about one-quarter of the exposed population will be immune to norovirus infection. For rotavirus the main impact is in children under 6 years who have no immunity. While a low dose of *Campylobacter* may have a high probability of infection, the Teunis et al. (2018) model predicts illness is only likely to occur at very high doses. In the absence of specific information on the probability of illness given infection, it is usually assumed that all infections will result in illness. When interpreting the results of a QMRA, it is important to consider that dose-response models are not based on the response of children or other vulnerable groups. Epidemiological studies of disease outbreaks have also been used to provide information on dose-response relationships; however, the exposure dose may be difficult to determine (Teunis et al., 2018).

The dose-response models used in AGWR (NRMMC et al., 2006) were based on Haas et al. (1999) for rotavirus, *Salmonella*, and *Campylobacter* and the model for *Cryptosporidium* was taken from Messner et al. (2001), whereas WHO (2006) used dose-response models from Haas et al. (1999) for all pathogens.

QMRA modelling is a complex activity and needs to be undertaken by a specialist, so the details of the different models are not presented here. However, some examples of dose-response models that have been applied since 2006 are:

- *Campylobacter* (Teunis et al 2018),
- norovirus (Messner *et al.*, 2014; Teunis et al 2008a),
- *Escherichia coli* O157 (Teunis et al 2008b)
- *Cryptosporidium* (McBride et al 2013)
- adenovirus types 4, 7, 16 (Teunis et al., 2016).

However, some modellers select the pre-2006 models (e.g. models for *Campylobacter* and *Giardia* developed by Rose and Gerba et al. (1991).

Different dose-response models can give different estimates of risk for the same virus. Norovirus models consider the virus to be aggregated or non-aggregated (Messner et al., 2014; Teunis et al., 2008a). Gonzales-Gustavson et al. (2019) suggested that an aggregated model could underestimate the risk of norovirus and consequently used the non-aggregated model, assuming that the WWTP would remove larger (i.e. aggregated) particles. Simhon et al. (2020) compared the results from both norovirus models and reported that the disaggregated model increased the risk of infection by 2.5-3.0 log_{10}. Thus, an assumption of disaggregation is more conservative and, therefore, appropriate in the absence of specific information to the contrary.

4. AGRICULTURAL REUSE

4.1 INTRODUCTION

In a rural setting, wastewater may be used as a water resource for irrigating edible or non-edible crops, orchards, pastures, forestry or nursery plants. In New Zealand, wastewater of varying quality may also be irrigated as a disposal mechanism, as this is more culturally acceptable than discharging wastewater to water bodies. The irrigation of wastewater could have adverse effects on farm workers, their families and communities, as outlined in section 2. Potential health risks are managed by reducing the direct and indirect exposure of people to pathogens. This can be achieved through different levels of wastewater treatment to remove pathogens, in combination with preventive barriers. These may include consideration of whether the food from the irrigated area is consumed raw, cooked or processed, the potential for contamination of milk or animal meat, restricting access to irrigation areas, using withholding periods before sale of a product to allow pathogens to die-off and the proximity of residents to the irrigation area (EU, 2020; ISO, 2018; NRMMH et al., 2006).

International guidelines (reviewed in Leonard et al. 2023) can be applied to manage risks for common applications of wastewater reuse, but there may also be a requirement for an analysis of risks for a specific application at a specific site. For example, the potential impact of spray irrigation will depend on local conditions, with generic buffer zones potentially being inadequate. Thus, while AGWR (NRMMC et al., 2006) specifies generic buffer zones of 50 m, the European guidelines (EU, 2020) propose that specific site assessments should be undertaken for the 'protection of workers or bystanders'. QMRA is the most common method used to assess site-specific risks. While the reuse treated of wastewater for irrigation of crops producing foods consumed raw is an activity that is practised internationally, it is assumed in this review that this is unlikely to be common in New Zealand as, in Te Ao Māori (the Māori world view), food and human waste are kept separate (Pauling & Ataria, 2010). There is also likely to be an adverse perception of this activity by the general public. This means that it is more likely that recycled wastewater would be used on crops producing foods that are not eaten directly (e.g. orchards), foods that are further processed (e.g. cereals) or pastures grazed by animals that may produce food products.

Despite being an unlikely application, a brief overview of QMRA literature to assess the risks associated with applying recycled the production of foods consumed raw is provided in the event of accidental contamination of food products. However, the consumption of pathogens through the contamination of groundwater from irrigated recycled wastewater and the augmentation of groundwater and surface water sources by recycled wastewater are not covered. Where these activities occur near drinking water sources, they are managed under Ministry for the Environment guidelines for drinking water source protection (PDP 2018) and the Resource Management Act 1991 (RMA). Discharges of wastewater to water are also covered under the RMA and health risks associated with this activity should be assessed through site-specific assessments. Recreational water quality guidelines require site specific assessments for discharge of treated wastewater to water (MfE 2003). Aquaculture with recycled wastewater is also considered unlikely in New Zealand and therefore is not specifically covered in this review, although it is covered in WHO (2006) as this practice may occur in low and middle-income countries.

The usual route of exposure to wastewater reused in agriculture is ingestion, which is covered in section 3. However, as identified in ISO (2018) and EU (2020), aerosols can be potential health hazards in areas when the activities creating them occur close to people and may also adversely affect farm workers or rural communities that live nearby.

4.2 IRRIGATION OF PASTURE, WOODLOTS AND FOOD

4.2.1 Irrigation of pasture and woodlots

Restrictions on the reuse of wastewater for grazing animals are usually related to the prevalence of animal disease within a given country. Thus, there may be restrictions on wastewater irrigation for pig farming in some countries (EU, 2020; NRMMC et al., 2006) due to the potential risk of infection with *Taenia saginata*, which can infect humans through the consumption of undercooked or raw meat. In New Zealand, guidelines for the application of sewage effluent to land require withholding periods for wastewater irrigation to protect grazing animals from helminth infection (DH, 1992), and Health New Zealand advises not to irrigate wastewater on pasture grazed by cattle (HNZ, n.d.).

Irrigation may be considered as an option for wastewater reuse for pasture or forestry. Subsurface irrigation or drip irrigation present a lower potential for human exposure than spray irrigation, which produces aerosols and spray drift. Rather than applying set buffer zones as in AGWR (NRMMC et al., 2006), but air dispersion and QMRA modelling can be used to determine an appropriate risk-based buffer zone. For example, Courault et al. (2017) modelled the reduction in health risk due to changes in pathogen concentrations in the air at different distances from the source of irrigation for different wind speeds and found that there was a 3 $\log_{10}$ difference in risk between 100 m and 1000 m at low wind speeds (<1 m/s). At wind speeds of >1 m/s, the 95th percentile probability of infection over 365 days, exceeded the annual risk of infection target of $<10^{-4}$ pppy. In this analysis, no single exposure gave a mean annual risk that exceeded 10^{-4} pppy, but exposure for 8 hours/day for 365 days gave a mean annual risk of infection of $<10^{-3}$ pppy at 500 m. While this may be an overestimate, as it is unlikely that people would be working 365 days/year, these findings do highlight the importance of assessing health risk from multiple exposures to determine separation buffers rather than using a prescribed buffer distance. Courault et al. (2017) indicated that uncertainties in the model included the effect of seasons and the assumption of a constant concentration of virus in the air. While a health survey undertaken in the area did not find a strong association between wastewater reuse and illness (aside from itchy skin), there was some overlap with rotavirus peaks in the community and the irrigation periods during summer months.

Site-specific modelling by Mori & Smith (2023) that used climate data from Illinois obtained from 2017 to 2019 in a QMRA showed that distances of 1-2 km and 2-3 km were required for spray irrigation from low- and high-pressure sprinklers, respectively, to meet the mean health target of $<10^{-6}$ DALYs pppy for *Legionella* for a single exposure. The median annual risk of infection was never exceeded, but it should be noted that multiple exposures were not modelled.

QMRA was also used by Seis et al. (2022) to compare on-demand and continuous irrigation over the summer period while changing the level of virus removal incrementally from 1 to 6 $\log_{10}$. At an annual risk of infection of 10^{-2} pppy (i.e. 1/10 of the cases of acute gastroenteritis reported in Germany), 4 $\log_{10}$ pathogen reduction was required at the WWTP for on-demand irrigation compared with 5 $\log_{10}$ pathogen reduction for continuous irrigation. However, this pattern was not evident where lower levels of log pathogen reduction occurred in the WWTP. With 6 $\log_{10}$ pathogen reduction, and on-demand irrigation the annual risk of infection ($<10^{-4}$ pppy) was met, and, unlike continuous irrigation would also occur over a shorter period and not overlap with the higher incidence of norovirus associated with the winter season.

These studies highlight the importance of site-specific modelling of aerosols and illustrate the effectiveness of mitigation measures. It is important to note, however, that modelling multiple exposures would be more realistic for both farm workers and residents.

4.2.2 Irrigation of foods not eaten raw

The health risk from irrigation with recycled wastewater is reduced when the wastewater does not come into direct contact with the food being consumed. Risk is further reduced if the food is peeled, cooked or further processed before consumption (e.g. lemons, wine and cereals).

Drip irrigation of wastewater in orchards or vineyards potentially has less inherent risk, as the wastewater does not come in direct contact with the food, although potential contamination of groundwater or surface water also needs to be considered. There must be no ponding, as this could potentially present a risk to workers, be tracked out of the orchard area or result in surface runoff, thereby impacting people not working in the irrigated area and the environment.

Effects such as microbial penetration of the roots, stems, leaves and plant tissues were examined by Perulli et al. (2021) using treated wastewater containing *E. coli* concentrations that were consistent with Italian regulations for the irrigation of orchards (<10 CFU/100 mL). This showed that there was an increase in concentration of *E. coli* in the shoots over one year. The authors also undertook a laboratory study of translocation to other parts of the plant but did not find evidence of translocation to the aerial parts of the tree after 30 days, even though some colonisation of the roots had occurred epi- and endophytically.

4.2.3 Irrigation of foods eaten raw

Although it is unlikely that recycled wastewater would be used in New Zealand for the production of food that is consumed raw, without peeling, or further processing this section highlights the risks in doing so, as determined using QMRA modelling, and provides some examples to illustrate the health risks identified using different QMRA approaches.

Inputs to a QMRA model can vary depending on the:

- type of vegetable and how they grow (e.g. lettuce, broccoli, cabbage, tomato, olive)
- water-retaining characteristics of the vegetable (e.g. iceberg lettuce is more open than a butternut lettuce, and cucumber retains less water than broccoli)
- local consumption rate of the vegetable
- type of irrigation used in the experiment (submerged in recycled wastewater, irrigated from above or drip irrigated)
- target pathogen
- analytical method used to enumerate the target pathogen (i.e. culture, which shows the pathogen is infectious, versus PCR techniques)
- washing vegetables
- holding period before consumption (i.e. die-off rate of the pathogen).

The risk assessment for the contamination of raw foods by pathogens was initially based on lettuces, which are popular and consumed raw. The initial laboratory procedure for contamination was developed by Shuval et al (1997, cited Makkaew et al., 2016)) and involved submersion of lettuces in contaminated water. Makkaew et al. (2016) validated this submersion technique through their finding that similar concentrations of *E. coli* occurred on lettuces grown under spray irrigation in field trials in South Australia (at weeks 4–6) and in laboratory studies where lettuces were submerged several times in contaminated water. The field trial data showed that *E. coli* concentrations on uncovered and covered lettuces was variable in the two days after spray irrigation and depended on the concentration of *E. coli* in the recycled wastewater and the weather conditions. At weeks 5 and 6 concentrations at Day 1 were higher than on the day of irrigation and less *E. coli* die-off occurred under plastic covering. It was proposed that this was due to the plastic providing protection from UV. As weather conditions are variable more than a two days withholding period would be necessary. No contamination was found on lettuces at harvest (6 weeks) when drip irrigation was used (Makkaew et al., 2016), but, Fonseca et al. (2011) did find *E. coli* K-12 on the leaves of lettuces up to 3 days following subsurface irrigation in late season, but not early season. It is noted that the concentrations of *E. coli* were high, around 10^6/100 mL.

Oliveira et al. (2012) studied the die-off of *E. coli* O157 on lettuces and soil from two irrigation events four weeks apart, using surface soil irrigation and direct sprinkler irrigation. It is noted that the concentration of *E. coli* O157 used in these experiments was much higher than is found in wastewater (10^9 CFU/100 mL). Using these high concentrations of *E. coli* O157, surface soil irrigation resulted in more soil contamination than sprinkler irrigation with low levels of contamination of the lettuce leaves. The reverse was found with sprinkle irrigation. There was also a seasonal effect, as reported by Fonseca et al (2011). *E. coli* O157 was undetectable four weeks after the irrigation event during spring, but remained detectable on the outer leaves at four weeks in the autumn experiments. This study also highlights the potential effect of environmental conditions.

Field trials also allow the effect of rainfall to be assessed. Rainfall was associated with increased concentrations of *E. coli* in spray-irrigated crops, which was attributed to soil splash but did not affect concentrations where drip irrigation was used, which was attributed to the small wetted area (Makkaew et al., 2016).

Verbyla et al. (2016) compared the ratio of *Giardia* and *Cryptosporidium* in water with irrigated soil and found that these pathogens accumulate in the soil. Therefore, the effects of long-term irrigation and pathogen accumulation should also be taken into account in QMRAs.

Vegetable consumption rates can differ markedly by country (e.g. more lettuce is consumed in China than Australia; Mok & Hamilton, 2014) and between seasons (e.g. more salad vegetables in summer than winter). Where all other parameters were equal Makkaew et al. (2016) showed that the QMRA model was more sensitive to the variation in consumption rates rather than the water retained by the vegetable. Therefore, when assessing the relevance of a given study to New Zealand conditions, it is important to also consider the consumption rates of raw vegetables (Barker, 2014; Makkaew et al., 2016).

The type of vegetable included in the QMRA is also important, as water retention varies. While iceberg lettuce has traditionally been the raw vegetable studied, Mok & Hamilton (2014) determined water retention vales for green oak lettuce (a more open lettuce), Chinese chard, Chinese broccoli and Chinese flowering cabbage in field trials in South Australia using overhead irrigation. Their results showed that the reuse of secondary treated wastewater did not meet the health targets of median annual probability of infection ($<10^{-4}$) or $<10^{-6}$ DALYs for any of these vegetables, even with washing, supporting the lower *E. coli* criteria set out in AGWR (NRMMH et al., 2006) rather than those specified by WHO (2006). Sensitivity analyses identified the concentration of rotavirus as the main parameter, with water retention by the vegetables and decay rates being minor factors.

Norovirus has generally replaced rotavirus as the basis for viral QMRAs since the introduction of a vaccine for rotavirus infections. However, Mok & Hamilton (2014) conducted a QMRA on rotavirus using data from a secondary WWTP in Beijing, as immunisation rates for rotavirus infection are low in China. It may also be useful to include rotavirus in site-specific QMRAs in New Zealand for places where immunisation rates are low and non-disinfected wastewater may be discharged to land as a wastewater disposal method. An advantage of using rotavirus is that is can be cultured. Mok & Hamilton (2014) used the 1 to 1000 ratio of culture (infectious):qPCR determined by Havelaar and Melse (2003, cited Mok & Hamilton, 2014) as an input for their model.

Mok et al. (2014) modelled the health risks from norovirus infection and included additional treatment of the wastewater from a secondary treatment plant. An extensive range of vegetables was included in their model (green oak lettuce Chinese broccoli, cabbage, bok choy, choy sum, gai lan and cucumber) using data from Hamilton et al. (2006) and Mok & Hamilton (2014). The concentration of norovirus in raw sewage was estimated from faecal shedding rates (section 3.2.1) and literature data was used for norovirus log removal in a waste stabilisation pond, enhanced chemical sedimentation, mechanical UV and chlorination (NRMMH et al., 2006). The model included the accumulation of viruses in vegetables from daily watering while applying a field die-off rate of 1 log_{10}/day for seven days obtained from the literature. The QMRA confirmed the findings of a previous study that wastewater from a WSP did not give sufficient pathogen removal to meet the health target of $<10^{-6}$ DALYs for a wide range of vegetables eaten raw, nor did the additional treatment or disinfection. A sensitivity analysis identified virus removal as the most sensitive parameter, with other wastewater treatment plant parameters, shedding rate and consumption rate being moderately sensitive contributors to risk estimates. WSPs achieve variable log pathogen reduction levels (1-3.5 log_{10}; NRMMC et al., 2006), and as further log pathogen reduction was required to achieve 6.5 log removal of virus for irrigation of food eaten raw (NRMMC et al., 2006), non-treatment options (e.g. withholding periods, washing vegetables) were not considered suitable to meet health targets. Furthermore, while additional wastewater treatment scenarios such as sedimentation and disinfection reduced the risk, the median risk still did not meet the DALY health target of 10^{-6} pppy, except for cucumber. The results of this more detailed model are contrary to the WHO guidelines (WHO, 2006), which assessed that WSP effluent would meet the health target if vegetables were washed and a withholding period was used before consumption.

In a separate study, Barker (2014) conducted a QMRA and applied three different methods to derive raw sewage norovirus concentration inputs for the model, as no Melbourne-specific norovirus data were available for raw sewage (see section 3.1.1). Where norovirus was estimated based on a mean literature value of 6.3 log_{10} GC/L, the annual DALY value met the WHO target of $<10^{-6}$ pppy. A minimum log reduction value of 6 and maximum of 8 or 11 was used for the WWTP, based on values given in AGWR (NRMMC et al., 2006), and a literature value was used for virus removal by mechanical UV. The model risk output was most sensitive to the virus log removal and raw sewage norovirus concentration either as a shedding rate (epidemiological model) or raw sewage concentration (analytical data models). The sensitivity analysis also highlighted that there was a 5 log_{10} range in virus removal when a lagoon was included in the treatment train.

Preventive measures such as washing vegetables and the application of a withholding period were not included as preventive measures in AGWR for the consumption of raw food due to the difficulty in ensuring the practices occurred (NRMMC et al., 2006). Barker (2014) collated published data on washing food to remove a range of viruses and noted the high variability in removal rates. Of six studies published since 2006, all of which were based on using tap water at commercial rather than domestic washing rates, the log removal rates ranged from 0.23 log_{10} for a surrogate norovirus (murine norovirus) on romaine lettuce (Predmore & Li, 2011) to 2.25 log_{10} for NoV GI and NoV GII on basil, although most products tested only had a log reduction of less than 1.5 log_{10} (Butot et al., 2008). Additionally, Barker et al. (2014) derived values for the domestic washing of vegetables using responses from a 1998 food safety survey reported by Mitakakis et al. (2004, cited by Barker et al 2014), which included responses from over 500 Melbourne homes. To meet health targets, Troldborg et al. (2017) illustrated that withholding periods could vary from as low as 2.3 days, to 23 and 46 days for norovirus and *Cryptosporidium,* respectively, depending on the decay rate used. Mok et al. (2014) applied a viral decay rate of 1 log_{10}/day to determine withholding periods, based on earlier work using *Bacteroides fragilis* by Pettersen et al. (2001; 2002 cited by Mok et al., 2014). More recent viral decay rates are available in the review by Boehm et al. (2019).

5. URBAN AND NON-AGRICULTURAL INDUSTRIAL REUSE

There are a wide range of urban wastewater reuse activities, ranging from firefighting to making concrete to the irrigation of public and private amenities, such as parks, gardens, sports fields and golf courses. Treated wastewater may also be reticulated to houses for reuse for irrigation or toilet flushing. An overview of international guidelines for the urban reuse of wastewater is provided in Leonard et al. (2023). AGWR (NRMMC et al., 2006) indicates that site-specific risk assessments should be undertaken for activities such as cooling tower water, and the European Union (EU) proposes site-specific assessments of health risks for spray irrigation, as discussed in section 4.

Residential bystanders could be exposed to reused wastewater for a small amount of time each day or every few days (e.g. through home gardening or participating in a recreational activity near reused wastewater, such as on a golf course or adventure park). However, workers are likely to be more at risk as they are required to be present eight hours/day.

In this section, recent literature on wastewater reuse for irrigation, toilet flushing and cooling towers is provided, as these are the most likely activities associated with urban wastewater reuse and cover most of the issues. One study on firefighting is also presented. The methodology in AGWR can be used to assess other activities, as proposed by ISO (2018).

5.1 AMENITY IRRIGATION

In urban areas, treated wastewater may be used to irrigate public or private amenities, such as sports fields, parks, gardens or golf courses. The method of irrigation may mitigate potential health risk. For example, subsurface irrigation reduces exposure to wastewater because the soil provides a preventive barrier between people and treated recycled wastewater, and drip irrigation reduces the exposure from spray drift or aerosolisation. For surface or spray irrigation which has greater potential for human contact, high levels of treatment at the WWTP are required to meet the wastewater quality guidelines in AGWR (NRMMC et al 2006).

The examples from the literature discussed below highlight how variation in input variables to QMRA models (e.g. exposure time, ingested volume or dose-response data) can affect the outcomes. Modelling of the risks of Legionellosis are also presented, as *Legionella* was not considered in AGWR (NRMMC et al., 2006).

The application of new models and new data can change recommended water quality criteria. A microbial risk assessment by Troldborg et al. (2017) compared different scenarios for amenity irrigation to understand the impacts of treatment and restricting access. This study applied a fuzzy logic model due to the large amount of input data, some of which was highly uncertain. Unlike AGWR, it also used a range of wastewater ingestion volume values and included dermal absorption and soil ingestion. The results indicated that *Cryptosporidium* and norovirus were potential infection risks (>10^{-4} pppy) if a person spent an average of 2.5 hours in a park during irrigation periods for 50 days of the year. This scenario requires the log pathogen reduction values in the WWTP to be higher than given in AGWR (NRMMC et al., 2006) to meet the health target. Different scenarios were also explored in the model with limited exposure (e.g. restricting access was found to be an effective mitigation measure). The authors proposed the use of UV mechanical treatment for the removal of *Cryptosporidium*, as chlorination is not effective against this pathogen.

Chhipi-Shrestha et al. (2017) modelled the *E. coli* criteria required for lawn and park irrigation to meet Canadian guidelines by applying a ratio of pathogenic *E. coli* to total *E. coli* in Canadian wastewater. From the model, they proposed a median criterion of 'not detected' and a maximum of 1 or 2 CFU/100 mL in five samples, which is 2 $\log_{10}$ less than the AGWR guideline (NRMMC et al., 2006). The model was most sensitive to the pathogenic *E. coli* ratio.

Legionella was not a target pathogen in AGWR (NRMMC et al., 2006) but has the potential to be a health risk, as discussed in section 3.1.3. Hamilton et al. (2018) assessed the potential risk of *Legionella* infection from spray irrigation in residential areas using QMRA, based on data from Johnson et al. (2018). In this model, the residential exposure and frequency inputs were 81-99 days/year for 1 hour/day. The results could also apply in a rural setting adjacent to farmhouses or recreational areas. Spray irrigation was assessed using conservative meteorological conditions that promote dispersion (wind speed 7 m/s, 25 km/hour) and a relative humidity (RH) of 65%. Culture, qPCR and a modified qPCR method that included an incubation step to better represent infectivity were used to measure the concentrations of *Legionella*. A light activity breathing rate was also used. To achieve the target annual risk of infection of $<10^{-4}$ pppy, setback distances of >75 m and 625 m were needed for risks based on median concentration values for culture and qPCR, respectively, while setback distances of 1025 m and approximately 10000 m were required at the 95th percentile for culture and qPCR, respectively. The health target for clinically severe infection was not met at distances less than 225 m for qPCR but was always met for samples analysed by culture.

The main difference between incidental and occupational exposure is the duration of the exposure. Hamilton et al. (2018) defined occupational exposure as 255 days/year and 8 hours/day and found that the associated setback distances to achieve health targets were significantly higher than calculated for incidental exposure. To meet the annual risk health target, the theoretical setback distances were approximately 1000 m and 5000 m using median concentration values for culture and qPCR, respectively, and 5000 m and more than approximately 10,000 m at the 95^{th} percentile *Legionella* concentrations for culture and qPCR, respectively. The median annual clinically severe infection residential population risk was always $<10^{-4}$ pppy at the minimum setback distance modelled (<75 m) for all assays. However, to meet the health targets at the 95^{th} percentile annual clinical severity population risk a setback distance of 1225 m was required using qPCR. Sensitivity analysis showed that the concentration of *Legionella* was the primary parameter influencing risk, but the sprinkler flow rate and efficiency of aerosol formation also had an effect. The results of this study also highlighted that the analytical method is critical, as *Legionella* concentrations determined by qPCR resulted in greater setback distances than concentrations determined by culture.

5.2 GOLF COURSES

Due to their high demand for water, the irrigation of golf courses with recycled wastewater is common in the Mediterranean basin (Salgot et al., 2012), USA (USEPA, 2012), Australia (Radcliffe & Page, 2020) and Canada (Simhon et al., 2020). Criteria for water quality and verification requirements have been defined in EU, state or national guidelines and require a high level of wastewater treatment.

In the absence of epidemiological data, Simhon et al. (2020) used QMRA to assess the risk of norovirus infection from golf course irrigation using WWTP data from Ontario, Canada, where national guidelines require treated water to be disinfected and held in a lagoon for 60 days. The QMRA used norovirus data from monthly sampling over one year at five WWTPs, and exposure was based on daily use of the golf course in the morning, when solar radiation and temperatures are lower, which would reduce norovirus die-off. Wastewater ingestion amounts were higher, at 6 mL, compared with the volume used in AGWR (NRMMC et al., 2006). Simhon et al. (2020) based their assessment on the risks of norovirus infection and reported that, with secondary treatment, disinfection (mechanical UV) and lagoon storage, the median daily probability of infection by norovirus (GI and GII) met the health target of $<10^{-4}$ pppy for early morning golfers. However, exceedances of the health target could occur if norovirus particles were assumed to be disaggregated, no immunity was assumed for the affected population and the other treatment trains were used (secondary treatment plus UV or secondary/tertiary treatment with chlorination). The treatment train that included secondary treatment, disinfection with mechanical UV treatment and 60 days of lagoon storage had the highest risk of infection, being 2-3 $\log_{10}$ greater than for the other treatment trains.

Similarly, Seto et al. (2018) undertook a QMRA using data from the WWTP at Vacaville, California, where there is secondary treatment with chlorination and de-chlorination, with removal rates of $>10^{3}$ $\log_{10}$ for norovirus. They found that the risks associated with norovirus, *Giardia* and *Cryptosporidium* in irrigated wastewater were one order of magnitude less than the annual infection health target of $<10^{-4}$ pppy. The removal of norovirus in the WWTP was better than reported by Simhon et al. (2020), who found poor removal of norovirus with chlorination at rates used to remove the *E. coli* indicator bacteria. The studies highlight the variability in WWTP pathogen removal and the need for local data to assess risks from irrigation with wastewater.

Chhipi-Shrestha et al. (2017) looked at setting a guideline value for *E. coli* in treated wastewater for public park irrigation based on the pathogenic *E. coli* ratio measured in British Columbia wastewater. They recommended a guideline of no detectable *E. coli*, with a maximum concentration of ≤2 *E. coli*/100 mL, which is lower than recommended in AGWR (NRMMC et al., 2006).

5.3 RETICULATED WASTEWATER FOR LAWN IRRIGATION AND TOILET FLUSHING

Recycled wastewater can be reticulated in residences as an alternative water source for toilet flushing and irrigation of gardens. Risk assessments and other exposure assessment methodologies have indicated the possibility of infections occurring from reticulated recycled wastewater (Hamilton et al., 2018; Hines et al 2014). Reticulation also introduces the potential of a new exposure pathway not considered in the risk assessments cited above,such as cross connections of recycled wastewater with drinking water. AGWR (NRMMC et al. 2006) proposed that 1 in 1000 connections could be cross connections.

No epidemiological data have been reported linking adverse human health outcomes to toilet flushing, but this could be due to high levels of treatment. For example, at Rouse Hill, Sydney, recycled reticulated wastewater is treated to achieve 8 and 10 $\log_{10}$ removal of enteric viruses and *Cryptosporidium*, respectively, so that it would not be a health risk even if directly ingested (Parliament of Australia, n.d.).

Hamilton et al. (2018) assessed the annual health risks from *Legionella* in recycled wastewater used for toilet flushing using data from Johnson et al. (2018). This study used three different models of aerosol generation from flushing toilet pans and subsequent inhalation and three different methods of *Legionella* analysis (culture, qPCR and modified qPCR). They found that the median risk exceeded the US target of a risk of <10^{-4} infections pppy for all three risk models when *Legionella* was analysed by qPCR, but only Risk Model 1 exceeded the health target when *Legionella* concentrations were determined by culture. Risk Model 1 was also the only model where the 95th percentile for severe clinical infection (pneumonia and hospitalisation) was exceeded, again based on *Legionella* concentrations determined by qPCR. The median risk with the modified qPCR analytical technique, which accounted for *Legionella* that may be present but not in a culturable state, was higher than the risk determined by culture but lower than the risk determined by qPCR. Sensitivity analysis showed that the concentration of *Legionella* was the primary determinant of risk. The partitioning coefficient and aerosol concentration were the next most important factors in risk models 1 and 2, respectively. Exposure time, dose response variable and exposure frequency were also important factors.

Chhipi-Shrestha et al. (2017) undertook a QMRA for lawn irrigation with recycled wastewater and included the ingestion of recycled wastewater from plant contact as well as accidentally irrigation the recycled wastewater. From this, they calculated a recommended median and maximum *E. coli* concentration of not detected and <1 *E. coli*/100 mL, respectively, based on the prevalence of pathogenic *E. coli* in Canadian wastewater. This is consistent with AGWR (NRMMC et al., 2006). However, the equivalent *E. coli* criteria for toilet flushing and use in the laundry were more stringent than in AGWR (NRMMC et al., 2006) with the median value of < *E. coli* detection limit and the maximum value of ≤3 *E. coli*/100 mL.

5.4 INDUSTRIAL USES

Although cooling towers have been implicated in outbreaks of legionellosis, and wastewater is a known source of *Legionella* (Caicedo et al., 2019; Johnson et al., 2018), no outbreak has been reported which identified wastewater reuse as the source of the outbreak.

Hamilton et al. (2018) undertook a QMRA for cooling tower mist inhalation, using data from Johnson et al. (2018). They found that the higher the wind speed, the greater the distance that the plume dispersed. They selected a wind speed of 7 m/s and a relative humidity of 65% as conservative values for modelling. For a 10 m high tower, the median annual risk of clinical infection for residential populations was above the health target at setback distances of <500 m for culture and <3500 m for qPCR, but at 95th percentile annual risk values, setback distances would need to be >5000 m for both culture and qPCR. The annual risk of clinically severe infection from exposure would require setback distances of 1000 m for qPCR, but the minimum setback distance met the health target using culture assays.

For occupational exposure, the risk exceeded the acceptable risk for infection at approximately 1000 m using culture assays and >5000 m for qPCR for the median annual risk, and at >5000 m for both culture assays and qPCR for the 95th percentile value. The median annual risk of severe clinical infection was always within the target for both culture and qPCR, but a distance of 2500 m was required to meet the 95th percentile when qPCR was used. Hamilton et al. (2018) determined that even at the minimum setback distance, the health target was met for the annual risk of severe clinical infection, at the 95th percentile.

When the height of the cooling tower increased to 100 m, the health target was met for the median annual risk of infection but was not met for the 95th percentile annual risk of infection between approximately 1000 m and >5000 m using both culture and qPCR assays. The health target for severe clinical illness was met for all distances modelled for all analytical techniques.

The concentration of *Legionella* was the primary determinant of risk, and water flow rate, dose response and cooling tower drift efficiency were the next most influential. It was also found that the risk could be reduced by 1–1.5 $\log_{10}$ if the efficiency of the drift eliminator was increased. These findings indicate that *Legionella* in cooling towers is a potential risk to human health and modelling is required to determine the magnitude of this risk.

Chhipi-Shreshta et al. (2017) undertook a QMRA to derive *E.coli* criteria that would protect health when wastewater was reused for firefighting and vehicle washing using the pathogenic *E. coli* ratio discussed in section 5.1. The derived criteria were median and maximum values of <1 *E. coli*/100 mL.

6. DISCUSSION AND SUMMARY

6.1 DISCUSSION

The purpose of this review was to identify literature published since 2006 that would provide information about health risks associated with the reuse of treated wastewater to support health submissions on regional rules or plans for resource consent under the RMA.

A review of epidemiological studies found that few studies have been undertaken in high-income countries. However, as non-disinfected secondary wastewater may be used in New Zealand as a means of wastewater disposal rather than reuse, the results of studies in low- and middle-income countries were discussed to highlight the risks to human health. In these studies, occupational exposure and having a person within a household with occupational exposure were identified as risk factors for gastrointestinal illnesses (Dickin et al., 2016 Adegoke et al. 2018). These two studies also acknowledged the contribution of other factors for illness, such as poor sanitary conditions and lack of access to clean drinking water.

In the absence of epidemiological studies on many types of recycled wastewater use, QMRA modelling has been used to determine the risk. Many of the inputs to the QMRA models were highly variable or highly uncertain, such as the concentration of pathogens in raw wastewater (Barker, 2014; Makkaew et al., 2016), the log pathogen reduction in WWTPs, especially from WSPs, and dose-response models, even when the same pathogen is used (Simhon et al., 2020). Sensitivity analyses reported for the various QMRAs confirmed that these parameters were more important than other variables, such as exposure from the amounts of vegetables consumed (Barker, 2014; Mok & Hamilton, 2014) or the (low) volumes of recycled wastewater ingested from consuming vegetables (Mok & Hamilton, 2014; Simhon et al., 2020).

Norovirus is the most commonly targeted viral pathogen, and a review of some of the literature highlighted differences in the concentrations of norovirus in wastewater between countries (Eftim et al., 2017), within WWTPs, with up to a 6 $\log_{10}$ difference within a single WWTP (Seis et al., 2022), between seasons with a 1 $\log_{10}$ difference in mean concentrations reported by Eftim et al. (2017) and between years with a one log difference determined by Seiss et al. (2022). The incidence of gastroenteritis can also vary by 1 log between countries (Barker, 2014). A strong correlation was found between reported incidences of NoV GII infection in a community and the concentrations measured in raw sewage (Seis et al., 2022). In the absence of local analytical data, faecal shedding rates have been used to calculate the concentration of norovirus in raw wastewater, but values calculated using this method differed from the literature by 1 $\log_{10}$ (Mok et al., 2014) and 3 $\log_{10}$ (Barker, 2014). While shedding rates can be adjusted for the volume of wastewater produced locally, they may over, or under, estimate the norovirus concentration when based on studies using small sample size as reported by Atmar et al (2008). In addition, analytical data from raw wastewater may underestimate concentrations due to low recovery rates. Therefore, applying a correction for recovery rates is important where analytical data are used.

These studies highlight the difficulty in determining a concentration for norovirus in raw wastewater and the importance of collecting local data on pathogen concentrations. and including virus recovery efficiency when reporting the data. As well as norovirus, the risk from *Cryptosporidium* in recycled wastewater could present a potential health risk for irrigation and consumption of food, as it is not removed by chlorination (Troldborg et al., 2017).

These studies highlight the and the importance of obtaining country-specific data. Concentrations in raw wastewater may exhibit 1 to 6 $\log_{10}$ variation due to seasonality and/or between-year differences, and the volume of wastewater produced per person and industrial contributions to a WWTP will also vary.

Log pathogen reduction values in WWTP also have a high level of uncertainty. At the concentrations of chlorine that are used to reduce the indicator bacteria *E. coli*, Simhon et al. (2020) reported low efficacy against norovirus. Numerous studies showed that *E. coli* is not a good indicator of the concentration of pathogens in treated wastewater (Bailey et al., 2018: Benjamin et al., 2013; Moazeni et al., 2017; Simhon et al., 2020; Verbyla & Mihelcic, 2015). Even viral surrogates, such as MS2 coliphage, may be poor indicators when WSPs are used for treatment (Verbyla & Mihelcic, 2015).

WSPs are commonly used in New Zealand where wastewater is being applied to land as a disposal technique, and it will be important to check that models assessing the health risks from irrigation of WSP treated wastewater include the impacts of spray drift and aerosols onto properties near these irrigation areas where food could potentially be grown, and people live or work. Of note is the finding that use of secondary treated wastewater did not achieve the health targets set by AGWR (NRMMC et al., 2006), or WHO (2006), when used for the irrigation of foods consumed raw (Makkaew et al., 2016; Mok & Hamilton, 2014; Mok et al., 2014). In addition, the *E. coli* criterion of 10,000 *E. coli*/100 mL (typical secondary wastewater treatment quality) in the WHO guidelines did not meet the DALY health target (Mok et al., 2014). Simhon et al. (2020) showed that health targets were still not met by additional treatment of secondary treated wastewater using disinfection, while the QMRA undertaken by Barker (2014) showed that a minimum of 6 $\log_{10}$ removal of pathogens was required to achieve health targets. For WSPs consistency in meeting these log pathogen reduction targets was an issue due to the inconsistency in wastewater quality (Makkaew et al., 2016).

The use of metagenomics in the analysis of wastewater and recycled wastewater infrastructure has highlighted the presence and accumulation of opportunistic pathogens such as *Aeromonas*, *Legionella, Mycobacterium* and *Pseudomonas. E. coli* is a poor indicator of the presence of these opportunistic pathogens, which have been reported as occurring more frequently than the indicator bacteria (Jjemba et al., 2010). While no outbreaks of Legionellosis have been attributed to irrigation using recycled wastewater, *Legionella* is potentially an important non-gastrointestinal pathogen in spray irrigation due to spray drift and aerosol production, which was not discussed in AGWR (NRMMC et al., 2006).

ISO (2018) and EU (2020) proposed that the effects of spray drift and aerosols on the health of people who live or work nearby are assessed. Recent literature presents examples of modelling for *Legionella* (Hamilton et al., 2018) and viruses (Courault et al., 2017) in aerosols, although Courault et al. (2017) does not include adenovirus (most strains of which cause respiratory infection). Air dispersion modelling adds more complexity to assessments of risk due to assumptions about air stability, temperature, wind speed, local topography and die-off rates from aerosolisation. Models have shown that the stronger the wind, the less dispersion there is and the further the plume will travel. This is an important consideration in New Zealand where wind conditions vary considerably within a day, within and between seasons, and between years and locations. Bare soil can also be an exposure pathway through aerosolisation (Girardin et al., 2016).

Since 2006, new models have been derived for dose-response modelling of *Campylobacter*, norovirus, *E. coli* O157, *Cryptosporidium* and adenovirus. Comparison of the models is beyond the scope of this review due to their technical complexity. The purpose of this review is to inform the scoping of QMRAs, not the detailed modelling, which is highly specialised.

The irrigation of pasture or plants is a potential use of recycled wastewater in New Zealand, but irrigation of foods eaten raw is likely to be culturally unacceptable to Māori and the public. Drip irrigation of orchards may be a potential reuse and, as it requires a very high standard of treatment, the potential contamination of food is low (Perulli et al., 2021), although the health risks from contamination of groundwater and surface runoff would need to be assessed. By contrast, spray irrigation has a potential health risk due to spray drift and aerosols as discussed above. QMRA studies highlighted the need for site specific modelling of spray irrigation to determine effective buffer zones. For example, Mori & Smith (2023) proposed 1 and 2 km buffer zones for low- and high-pressure spray irrigators, respectively, to protect against the risk of *Legionella* infection. This contrasts with the AGWR (NRMMC et al., 2006) approach, which specifies a generic 50 m buffer zone. Irrigation practices could also be modified from continuous irrigation to on-demand irrigation to protect farm workers from viral gastroenteritis (Seis et al., 2022).

Studies have examined the contamination of foods consumed raw by recycled wastewater and have shown that very high levels of pathogen reduction are required, with a minimum of 6 $\log_{10}$ and ideally 8–11 $\log_{10}$ reductions (Barker et al., 2013), to meet health targets. As well as uncertainty in the variables discussed above, data on other factors, such as the die-off rates of specific pathogens (rather than surrogates) is limited. This means that withholding periods before to allow pathogen die-off are uncertain. Proposed mitigation measures which rely heavily on the implementation of behavioural preventive measures, such as washing food or withholding periods are difficult to implement. All these uncertainties, and the clear link with illness from consumption of raw food irrigated with recycled wastewater with low levels of pathogen reduction, make this a high-risk activity.

Wastewater reuse in an urban setting may include the irrigation of amenities such as parks, golf courses and sports grounds, industrial reuse, and domestic reuse through reticulated systems. The effectiveness of WWTP in removing pathogens was modelled by Simhon et al. (2020) for use of recycled wastewater to irrigate golf courses. They identified that a daily golfer would have a health risk greater than the target ($<10^{-4}$ pppy) for all wastewater treatment trains except secondary treatment plus mechanical UV and 60 days storage in a lagoon based on data from Ontario, Canada. However, using Californian WWTP data Seto et al. (2018) found that the same health target would be met for norovirus with secondary treatment, chlorination and de-chlorination.

Legionella is also a potential risk from the reuse of wastewater for cooling towers. In the vicinity of cooling towers, people working outside, had a higher health risk than residents. The choice of Analytical method changed the setback distances. Culture and qPCR assays indicated that setback distances of 75 m and 625 m, respectively, are required for the protection of residents and setback distances of 1 km and 5 km, respectively, are required for protection from occupational exposure (Hamilton et al., 2018).

For reticulated use of wastewater, a very high rate of treatment is required, as AGWR (NRMMC et al., 2006) identified that cross-connections may occur at a rate of 1 in 1000 connections. This exposure pathway is often omitted from QMRA studies. Hamilton et al. (2018) used different analytical techniques for *Legionella* and showed that the ability to meet the health target depended on the model and the analytical technique. For example, for toilet flushing, the median risk was within the health target of $<10^{-4}$ pppy for only one model of air dispersion using culture but was always above the target for infection using qPCR, and similarly, clinically severe infection was only above the health target when qPCR was used for analysis.

The introduction of new target pathogens can change the log pathogen reduction required from that given by AGWR (NRMMC et al., 2006). For example, Gonzales-Gustavson et al. (2019) increased the target for virus removal in their QMRA using norovirus from a log pathogen reduction of 6 $\log_{10}$ in AGWR (NRMMC et al., 2006) to 7 $\log_{10}$ in order to meet health targets for irrigation of foods consumed raw. Similarly, Chhipi-Shrestha et al. (2017) used the pathogenic *E. coli* ratio in their QMRA and proposed lower *E. coli* criteria in the Canadian guidelines for amenity irrigation and golf courses than are presented in AGWR (NRMMC et al., 2006). The log pathogen reduction calculated by Troldborg et al. (2017) for *Cryptosporidium* was also higher than given in AGWR (NRMMC et al., 2006) for irrigation of public amenities .

6.2 SUMMARY

The purpose of the review was to identify literature published since 2006 that would provide information about the health risks from reusing treated wastewater to support health submissions on regional rules or plans for resource consent under the RMA.

There is very little in the literature on epidemiology as most high income countries where it is used extensively have high criteria for reuse of wastewater. No *Legionella* outbreaks have been attributed to wastewater reuse, but it is a potential risk as this pathogen accumulates in wastewater and recycled wastewater infrastructure.

As there is an immense amount of literature on many aspects of wastewater recycling we have looked at key areas. The report provides an overview of new knowledge on input data (pathogens, concentrations, removal, persistence, growth, aerosols as exposure pathway, dose-response models). It then looks at studies on reuses likely to occur in New Zealand such as irrigation and toilet flushing. It does include aspects of irrigation of poorly treated wastewater as it can be spray irrigated as a disposal to land option in New Zealand.

A review of QMRAs showed that there is a lot of variability and uncertainty in the input data that needs to be reported with the results to assess the health risk of a given activity. The key points were as follows:

- Studies should use local data for the concentrations of pathogens in raw sewage collected over different seasons and years.
- Studies should use log pathogen reduction values that reflect the efficiency of local WWTPs.
- Different dose–response models can give different risk estimates for the same pathogen.
- Indicator organisms used to assess log reductions in pathogens should reflect the type of pathogen, as their levels of resistance to treatment and environmental survival differ.
- *E. coli* concentrations do not always relate to pathogen concentrations, or pathogen removal, in treated wastewater.
- As proposed by EU (2020) and ISO (2018), spray drift and aerosols should be included in risk assessments with local conditions to set buffer distances, rather than using a generic setback distance.

- The human health risk from *Legionella* should also be included in risk assessments.
- While recycled wastewater is unlikely to be used to grow food that is eaten raw, the potential for spray drift onto food should be taken into account.
- Assumptions made in QMRA studies should be checked to ensure they are appropriate, as most results are most sensitive to parameters which may not be well characterised e.g. pathogen concentrations or removals in WWTP.

Recent QMRA models have highlighted that the WHO (2006) guidelines for wastewater treatment suitable for the irrigation of foods eaten raw are not protective. The use of new or updated data have also shown that lower log pathogen reduction values than are given in AGWR (NRMMC et al., 2006) may be required for the irrigation of public areas, including golf courses.

The literature shows that QMRA is a useful tool for assessing human health risk from recycled wastewater use and that local data improve the assessment. However, a sensitivity analysis of the inputs is critical, as there is a high level of uncertainty in key input data. QMRAs can assist in decision making, as they allow the effectiveness of different mitigation measures or interventions to be compared.

ABBREVIATIONS

AGWR	Australian Guidelines for Water Recycling
CFU	Colony-forming units
DALY	Disability-adjusted life year
DH	Department of Health
EPHC	Environment Protection and Heritage Council
ESR	Institute of Environmental Science and Research
EU	European Union
FIB	Faecal indicator bacteria
GC	Genome copies
GI	Geongroup I
GII	Genogroup II
HNZ	Health New Zealand
ICC-PCR	Integrated cell culture - Polymerase chain reaction
ISO	International Organization for Standardization
MPI	Ministry for Primary Industries
NHMRC	National Health and Medical Research Council
NRMMC	Natural Resource Management Ministerial Council
PCR	Polymerase chain reaction
PDP	Pattle Delamore Partners Ltd
pppy	Per person per year

QMRA	Quantitative microbial risk assessment
qPCR	Quantitative polymerase chain reaction
RMA	Resource Management Act 1991
UV	Ultraviolet
WHO	World Health Organization
WSP	Water stabilisation pond
WWTP	Wastewater treatment plant

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