Human health risks for *Legionella* and *Mycobacterium avium* complex (MAC) from potable and non-potable uses of roof-harvested rainwater

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Running title: QMRA of opportunistic pathogens in roof-harvested rainwater

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ABSTRACT

A quantitative microbial risk assessment (QMRA) of opportunistic pathogens *Legionella pneumophila* (LP) and *Mycobacterium avium* complex (MAC) was undertaken for various uses of roof-harvested rainwater (RHRW) reported in Brisbane, Australia to identify appropriate usages and guide risk management practices. Risks from inhalation of aerosols due to showering, swimming in pools topped up with RHRW, use of a garden hose, car washing, and toilet flushing with RHRW were considered for LP while both ingestion (drinking, produce consumption, and accidental ingestion from various activities) and inhalation risks were considered for MAC. The drinking water route of exposure presented the greatest risks due to cervical lymphadenitis and disseminated infection health endpoints for children and immune-compromised populations, respectively. It is therefore not recommended that these populations consume untreated rainwater. LP risks were up to 5 orders of magnitude higher than MAC risks for the inhalation route of exposure for all scenarios. Both inhalation and ingestion QMRA simulations support that while drinking, showering, and garden hosing with RHRW may present the highest risks, car washing and clothes washing could constitute appropriate uses of RHRW for all populations, and toilet flushing and consumption of lettuce irrigation with RHRW would be appropriate for non- immune-compromised populations.

Keywords: Roof-harvested rainwater; opportunistic pathogens; quantitative microbial risk assessment (QMRA); *Legionella pneumophila*; *Mycobacterium avium* complex.

1. Introduction

Globally, harvested rainwater is used to supplement both potable and non-potable water supplies. In Australia, roof-harvested rainwater (RHRW) constitutes an important source of water for many households; in 2010, 32% of Australian households had a rainwater tank and rainwater tanks were the main source of drinking water for 13.6% of Queensland households (ABS, 2010). Queensland rainwater tank owners have reported numerous potable and non-potable uses for their RHRW, including drinking, cooking, clothes washing, showering, pool top-up, gardening, car washing, ornamental water features, toilet flushing, filling fish tanks, and pet washing (Hamilton et al., 2016). This indicates the potential for exposure to rainwater through numerous scenarios.

Previous studies have identified high concentrations of both enteric pathogens such as *Salmonella* spp., *E. coli*, *Campylobacter* spp., *Cryptosporidium* spp., and *Giardia* spp. (Ahmed et al., 2011; Ahmed et al., 2010; Crabtree 1996) and opportunistic pathogens such as *Legionella pneumophila* (LP), *Mycobacterium avium* complex (MAC), *Aeromonas hydrophila*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Acanthamoeba* spp. (Ahmed et al., 2014; Hamilton et al., 2016) in subtropical rainwater tanks. Opportunistic pathogens cause illness primarily in individuals with underlying health conditions, children, and/or the elderly. However, they are a growing cause of drinking water-associated disease outbreaks worldwide (Falkinham 3rd et al., 2015).

There is epidemiologic evidence of disease cases associated with enteric pathogens (Brodribb et al., 1995; Franklin et al., 2009; Koplan, 1978; Merritt et al., 1999; Murrell and Stewart, 1983; Simmons and Smith 1997) as well as opportunistic pathogens LP (Schlech III et al., 1985; Simmons et al., 2008) and MAC (Lumb et al., 2004) in RHRW. While drinking water guidelines used to determine the safety of Australian rainwater specify the non-detection of *E. coli* in 100 mL of water (NHMRC-NRMMC, 2011; WHO, 2004), there is no consensus about which end-uses of rainwater are appropriate with regards to opportunistic pathogen associated health risks. No study has assessed the full suite of potential rainwater uses for an opportunistic pathogen to make such a determination. This is especially important as treatment options are limited for rainwater tank owners and are typically limited to gutter protection, first-flush devices, or point-of-use filters. These options have limited efficacy for removing pathogens (Dobrowsky et al., 2015a; Dobrowsky et al., 2015b; Egodawatta et al., 2009; Jordan, 2008; Kus et al., 2010; Mendez et al., 2011; Reyneke et al., 2016).

Quantitative microbial risk assessment (QMRA) can be used for the purposes of estimating the human health risk associated with exposure to pathogens in environmental matrices using a process of hazard identification, exposure assessment, dose response, and risk characterization (Haas et al., 1999). Due to their direct linkage to RHRW-associated disease cases (Lumb et al., 2004; Simmons et al., 2008), global epidemiologic importance (Falkinham 3rd et al., 2015), and known occurrence in RHRW (Hamilton et al., 2016), LP and MAC were chosen as index opportunistic pathogens for a QMRA estimate. While there are over 50 species of *Legionella* and several are human pathogens, LP is the most common species that causes the severe pneumonia-like illness Legionnaires' Disease, as well as the less severe form of illness, Pontiac fever (Diederen, 2008; Muder and Victor, 2002). The rate of Legionellosis in Australia was 13 per million people in 2012, and eighty cases were reported in Queensland in 2015 (Australian Government Department of Health, 2016; Phin et al., 2014). MAC is a subset of non-tuberculous mycobacteria (NTM) that can cause skin and soft tissue infections or cervical lymphadenitis in immune-competent patients, disseminated infections in immune-compromised patients, and pulmonary disease in both healthy and immune-compromised groups (Falkinham 3rd, 1996). MAC were the most common pathogen in NTM isolates in Queensland in 2005, and most frequently identified isolate in NTM cases in the Northern Territory, Australia from 1989-1997 (O'Brien et al., 2000; Thomson et al., 2013), however NTM-associated illnesses are not reportable in Australia. MAC is comprised of 9 species (*M. avium*, *M. intracellulare*, *M. arosiense*, *M. chimaera*, *M. colombiense*, *M. marseillense*, *M. timonense*, *M. bouchedurhonense*, and *M. ituriense*) (Falkinham 3rd, 2013). The most human-relevant species and therefore the focus of this QMRA are *M. avium* (comprised of four subspecies: *paratuberculosis*, *avium*, *hominissuis*, and *silvaticum*), *M. intracellulare*, and *M. chimaera* (Hamilton et al., 2017).

Previous QMRA studies of RHRW have focused on enteric pathogens or LP, typically focusing on one or two exposure scenarios (Ahmed et al., 2010; de Man et al., 2014a; Fewtrell and Kay, 2007; Lim et al., 2015; Lim and Jiang, 2013; Schoen and Garland, 2015; Schoen et al., 2014). This has been partially due to the lack of dose response models for opportunistic pathogens such as MAC. A single previous assessment of exposure to MAC is available for treated tap water from a centralized distribution system (Rice et al., 2005). However, this study did not quantify health risks. A dose response model has since been developed for one MAC subspecies, *M. avium* subsp. *paratuberculosis* (MAP) (Breuninger and Weir, 2015), however the relationship between human

exposure to this subspecies and the development of health effects [it is postulated that Crohn's disease may be the health outcome for this pathogen (Pierce, 2009; Waddell et al., 2015)] is contentious and is therefore excluded from the current analysis. A family of MAC dose response models was recently developed for human-relevant species of MAC in environmental matrices, allowing for development of a population- and exposure-route specific QMRA for MAC risks (Hamilton et al., 2017). For LP, generally only inhalation or aspiration routes are considered relevant, with inhalation being the most common exposure route (Ellis, 1993).

The goals of the current study are therefore to assess the health risks from index pathogens LP and MAC by conducting a QMRA of multiple potential exposure scenarios with (1) inhalation exposure to LP and (2) inhalation and ingestion exposures to MAC.

2. Materials and Methods

2.1 Exposure models

In our previous study, Queensland rainwater tank owners reported using RHRW for drinking, cooking, clothes washing, showering, pool top-up, gardening, car washing, ornamental water features, toilet flushing, filling fish tanks, and pet washing (Hamilton et al., 2016) (Supplementary Table S1). In that study, LP and MAC were measured in 134 rainwater tanks from Brisbane, Australia. The use of RHRW for ornamental water features, filling fish tanks, and pet washing were reported by less than 2% of surveyed residents, and were therefore excluded from the current QMRA analysis. The remaining potential exposure pathways for LP and MAC are shown in Fig. 1. As reported in a previous study (Hamilton et al., 2017), exposure to MAC species can occur through: (1) the ingestion of food, water, or soil; (2) inhalation of water aerosols or soil dusts; (3) aspiration; or (4) iatrogenic exposure. Scenarios (1) and (2) were considered here as it is unlikely to use harvested rainwater in a medical setting, and limited information is available regarding aspiration rates and the amount of pathogenic material transferred to the lungs during each aspiration event. Exposure models for each scenario are summarized below and in Fig. 1.

It is noted that in addition to the importance of the exposure route, MAC is associated with different health endpoints depending on the population exposed (Hamilton et al. 2017). Ingestion routes of exposure are associated with cervical lymphadenitis in children or disseminated infection in immune-deficient individuals (Falkinham 2013). Immune-deficient individuals can be either adults or

children. Children over the age of 12 are rarely affected by MAC lymphadenitis, except when immunodeficiencies or disseminated disease are present (Lai et al. 1984), and cervical lymphadenitis is most common in children from ages 1 to 5 (Inderlied et al. 1993, Haverkamp et al. 2004, Wolinsky 1995, Lincoln et al, 1972, Hazra et al. 1999, Tebruegge et al. 2016). For this reason, MAC risks for the ingestion pathway were calculated separately for a general immune-deficient population and children ages 1-2, 3-5, and 6-12 based on differences in disease presentation, susceptibility to MAC, and exposure patterns. Despite similar child susceptibility to MAC from age 1 to 5, activity patterns were different depending on ages and thus two categories (1-2 and 3-5) were used. Inhalation exposures to MAC have been shown to be epidemiologically important for healthy, post-menopausal females or older males with pre-existing lung conditions and/or risk factors, however healthy individuals may also become ill (Field et al. 2004). Children may also be affected via the inhalation route, although this is less common (Freeman et al. 2009, Nolt et al. 2003). Additionally, skin and antibody test reactivity studies have indicated increasing levels of exposure with age and evidence of asymptomatic infection with MAC (Griffith et al. 2007). However, sufficient information is not available at this time to develop separate risk expressions for MAC respiratory infection on the basis of specific age range or susceptibility factors. Therefore, for the inhalation models, MAC infection risks were calculated for a general population rather than age- or immune status- separated populations. Legionnaires' disease has been reported in children (Luttichau et al. 1998; Aebischer et al. 1999; Campins et al. 2000), and a general population at risk was also considered.

2.2 Ingestion exposure models

As Legionellosis is contracted through inhalation or aspiration, ingestion exposure models apply only to MAC. The general process for ingestion of RHRW for a variety of applications for MAC can be described by equation (1). Additional factors and modifications for each scenario are described below and summarized in Table 2.

$$D_{i,j} = \frac{1}{R} C_{RHRW,MAC} V_{ing,j} \qquad (1)$$

Where $D_{i,j}$ = The daily dose of pathogen *i* (where *i* = LP or MAC) for exposure scenario *j* (where *j* = drinking, eating produce, etc.), C_{RHRW} = Concentration of MAC in RHRW, *R* = recovery efficiency (%), and V_{ing} = the volume of RHRW ingested per exposure event.

2.2.1 Drinking

A mean of 0.869 L per day and 95th percentile 2.717 L per day (US EPA, 2011a) was used to construct a lognormal distribution for daily drinking water intake for a general population. For children, drinking water exposure parameters reported for ages 1 to <2 years, 3 to <6 years, and 6 to <11 years were used for 1-2, 3-5, and 6-11 year-old child age groups, respectively (US EPA, 2011a). Parameters reported for ages 1 to <2 years and 2 to <3 years were used to fit overall 1 to 2 year parameters by generating 10,000 observations from each set of parameters (1 to <2 year and 2 to <3 year) which were then concatenated. The age brackets were weighted equally as the proportion of 1 and 2 year olds in the Queensland population was equivalent in 2015 (ABS, 2016). A new distribution was fit to the combined data.

Two scenarios, without filtration and with filtration (equation 2) were considered as some participants in our previous rainwater study (Hamilton et al., 2016) had under-sink point-of-use (POU) filters. Chlorine disinfection was not considered as only one participant of 134 households reported having ever used chlorine to disinfect their rainwater (Hamilton et al., 2016). The most common treatment method for those who reported treating their rainwater was filtration (29/134 participants) (Hamilton et al., 2016). For a POU filter used with rainwater systems, a previous study reported a 39% (0.4 log) reduction in *E. coli* bacteria (Jordan, 2008). A sand filter used with rainwater tanks was associated with a 99% (2 log) removal of bacteria (Ahammed and Meera, 2010). A uniform distribution for filtration of 0.4-2 log removals was used.

$$D_{i,j} = \frac{1}{R} C_{RHRW,MAC} V_{ing,j} 10^{-L} \qquad (2)$$

Where, L = the number of log removals for filtration.

2.2.2 Consumption of raw produce

The consumption of uncooked lettuce was chosen as the index scenario for eating produce irrigated with RHRW. The selection of lettuce was due to its high water retention compared to other crops because of its large and uneven surface area, its high probability of being eaten raw, high lettuce consumption compared to other produce crops by Australian populations, and short shelf life that bounds the possible time between harvest and consumption, especially in a subtropical climate (Ahmed et al., 2016). Contamination with MAC in lettuce can occur through processes of: (1) the irrigation water adheres to the outside of the plant and (2) MAC is internalized into plants (Kaevska et al., 2014). Internalization can occur through uptake through the roots, or through stomata or wounds present on the leaf surface (Hirneisen et al., 2012). These microorganisms would not be washed off

by the consumer as they are located inside the plant. To account for both of these processes (surface-attached and internalized *M. avium*), the total dose of MAC on the surface of lettuce and the MAC internalized in the plant are summed to arrive at the total dose (equation 3 - equation 5) (Sales-Ortells et al., 2014).

$$D_{MAC,surface} = \frac{1}{R} C_{RHRW,MAC} V_R 10^{-k_{f,s} t_f} 10^{-W} I B_a F_{CL} \ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{(Eqn. 3)}$$

$$D_{MAC,internalized} = \frac{1}{R} C_{RHRW,MAC} F_{int} V_R I B_a F_{CL} \ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{(Eqn. 4)}$$

$$D_{MAC,total} = D_{MAC,surface} + D_{MAC,internalized} \ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{(Eqn. 5)}$$

Where V_R = volume retained per gram of lettuce during irrigation (L per gram), I_a = gram consumed per kg-person per day for age range *a* where *a*= ages 1-2, 3-5, 6-12, or a general population (g/kg/day), B_a = body weight for each age range *a* of interest (kg), F_{CL} = fraction of population of interest that consumes lettuce, $K_{f,s}$ = in-field decay constant for MAC on the surface of lettuce (per day), W = log reductions due to washing of lettuce prior to consumption, $K_{f,int}$ = in-field decay constant for internalized MAC (per day), F_{int} = the internalized fraction of MAC in the irrigation water that is found in the leaves (in CFU per gram of lettuce), t_f = withholding time between harvest and consumption (days). Lettuce is assumed to be immediately consumed by the homeowner after harvest, and, therefore decay of MAC due to transport and/or storage of the lettuce is not considered.

Internalization has typically been reported for enteric bacteria or viruses in a variety of produce (Dicaprio et al., 2012; Solomon et al., 2002; Wei et al., 2011), however, only one report of internalization of *M. avium* in plants is available. Internalization rates were calculated from Kaevska et al., (2014) (Fig. 2 and 3) data from field studies with tomato plant leaves by computing (concentration recovered from leaves after surface washing) / (concentration of seeding inoculum) for lettuce. Internalized fractions ranged from 1.13×10^{-5} to 9.49×10^{-4} for *M. avium* subsp. *paratuberculosis* and 1.00×10^{-8} to 1.37×10^{-5} for *M. avium* subsp. *avium*. A uniform distribution using the more conservative *M. avium* subsp. paratuberculosis values was used. During field experiments conducted by Kaevska et al., (2014) over 8 weeks, concentrations of *M. avium* in leaves did not consistently increase or decrease between 3 (*M. avium* subsp. *avium*) or 4 (*M. avium* subsp. *paratuberculosis*) sets of experiments. For this reason, decay was not included in the model for internalized *M. avium*.

For the volume of water retained by lettuce during irrigation, Shuval et al., (1997) estimated a mean and standard deviation of 0.108 ± 0.019 mL per g for green oak lettuce planted in beds with

three staggered rows spaced 30 cm apart with fixed-set overhead sprinklers at 10 min intervals. It was assumed that microorganisms are homogeneously distributed in RHRW stored in tanks and 100% of the organisms in the retained irrigation water become initially attached to the lettuce surface.

Decay experiments for *M. avium* on the surface of plants were not available, however Cook et al., (2013) performed decay experiments for *M. avium* subsp. *paratuberculosis* exposed to silage exudates derived from grass and alfalfa. A decay *k* value of -0.0484 per day was reported. Removal of *E. coli* during washing with water of 0.3 ± 0.1 log (Holvoet et al., 2014) was assumed to be representative of removal of *M. avium* and *M. intracellulare.*

A previously reported consumption distribution for the general population was used for lettuce (Barker et al. 2013). Body weights for the general Australian population were computed by conducted weighted sampling of distributions simulated from each age range reported in the literature based on the age profile of the greater Brisbane area (ABS 1998, ABS 2016, CSIRO 2008). Australian data was not available in a format that could be separated for ages 1-2, 3-5, and 6-12 years. For this reason, lettuce consumption and body weight data from a US population was used for children 1-12 years old (USEPA 2011a).

2.2.3 Showering

A uniform distribution of water accidentally ingested per daily shower event was used of 58 µL - 1.9 mL (Ahmed et al., 2010). Information was not available for child-specific exposure, therefore these estimates were used for both child (ages 6- 12) and general population exposures.

2.2.4 Garden hose use

Estimates for accidental ingestion during use of a garden hose has been reported to range from 0.002 -1.9 µL (Ahmed et al., 2010), up to 1 mL (NRMMC-EPHC-AHMC, 2006), or 1.1 mL (Schoen et al., 2014). Irrigation of lettuce with recycled water was estimated to occur 90 times per year (NRMMC-EPHC-AHMC, 2006). Gardening was not considered for child-specific exposure scenarios.

2.2.5 Car washing

The total volume of water consumed during 10 min of car washing using a high-pressure spray device was recently estimated using cyanuric acid as a tracer of water ingestion. Among 26 participants, the accidental ingestion volume per car washing event was estimated to range from 0.06 to 3.79 mL (Sinclair et al., 2016). A monthly car washing frequency was assumed (Villarreal and Dixon, 2005). Car washing was not considered for child-specific exposure scenarios.

2.2.6. Pool top-up and bathing

Australian pool-owners reported using RHRW as well as drinking water to fill their swimming pools and all pools observed in the previous study were outdoor family pools (Hamilton et al., 2016). The distribution of the proportion of drinking water: RHRW is not known as pool sizes, tank sizes, and pool maintenance practices vary considerably. As a result, scenarios of 10, 50, and 90% dilution of RHRW with opportunistic pathogen-free water in pools were modelled (equation 6). Additional pathogen decay through mixing of RHRW with tap water with a chlorine residual was considered to be minimal and potential opportunistic pathogen removal due to pool filters was not considered.

$$D_{i,j} = \frac{1}{R} C_{RHRW} V_{ing} D \qquad (6)$$

Where *D* = dilution factor and *D* =100% for the bathing water scenario (100% rainwater).

An ingestion volume of 51.5 mL and standard deviation of 103.5 mL per swim (lognormal parameters μ = 2.92 mL, σ = 1.43 mL) for a combined population of children and adults was used (Dufour et al., 2006). For child ingestion during swimming in pools or young child exposure during daily baths (ages 1 to 5; in place of showers), the distribution for child swimming pool ingestion from Schets et al. (2011) for Dutch children <15 years was used. Information was not available to fit separate distributions to the separate child age groups of interest.

Limited information is available regarding the frequency of swimming pool use and no studies of swimming frequency from Australian freshwater pools could be found. Only the study from the Netherlands (Schets et al. 2011) reported swimming pool use frequency as 13-24 times per year on average (95% CI up to 65 days per year for adults and 91 days per year for children) for a five-month swimming season lasting from May 1 through October 1. Brisbane is coolest from June through August (Australian Government Bureau of Meteorology, 2016). Assuming that the Queensland swimming season takes place during a 9 month swimming season from September through May and that the rate of Netherlands pool swimming frequency is similar, the distributions for swimming frequency in swimming pools from Schets et al. were scaled to account for a 33% longer swimming season in Australia. The child distribution (<15 years of age) was used for all child age brackets due to lack of information on specific age ranges. To obtain parameters for a general swimming pool frequency distribution, distributions were simulated from the male, female, and child swimming frequency parameters (Schets et al. 2011) and weighted according to the portion of adults and children < 15 years of age in the greater Brisbane population (ABS, 2016). The distributions were

298 concatenated and a negative binomial distribution was fit to the resulting dataset. Using the modified
299 distributions, average and upper bound (95th percentile) point estimates for swimming frequency were
300 calculated.

301 *2.2.7. Toilet flushing*

302 For toilet flushing, estimates of the volume consumed per flush range from 0.01 mL (NRMMC-EPHC-
303 AHMC, 2006) to 0.3 mL (Schoen et al., 2014). A mean of 5 flushes per day was reported (Mayer and
304 DeOreo, 1999). Toilet flushing exposures were considered for child exposure models for ages > 3
305 years only as the average age of toilet training is approximately 3 years (Bloom et al. 1993). Child-
306 specific exposure information was not available, therefore the same distributions for number of
307 flushes were used for all age groups.

308 *2.2.8. Clothes washing*

309 An exposure volume of 0.01 mL 100 times per year is estimated for exposure to water used for
310 clothes washing (NRMMC-EPHC-AHMC, 2006). No aerosols were observed during clothes washing
311 experiments, therefore, only ingestion is considered for clothes washing (O'Toole et al., 2008a).
312 Clothes washing exposures were not considered for child-specific exposure models.

313 *2.3. Inhalation exposure models*

314 *2.3.1 Showering, garden hosing, car washing, toilet flushing*

315 Inhalation exposures were considered for both LP and MAC. However, the MAC health endpoints and
316 population at risk differs from ingestion exposures and as a result all inhalation models accounted for
317 pulmonary infection risks in a general population rather than adult- or child- specific pathways. LP
318 concentrations are parameterized slightly differently than MAC due to the small number of detects (*n*
319 = 4 positive out of 134 samples) in rainwater tanks in the study by Hamilton et al. (2016). Maximum
320 likelihood used for MAC is typically not appropriate for this high degree of censoring and thus a
321 binomial method was used. Equations 7 and 8 were used for showering, garden hose use, car
322 washing, and toilet flushing, accounting for the volume of aerosols of various size diameters that are
323 large enough to hold LP or MAC but small enough to deposit at the alveoli (1µm < diameter < 10µm).
324

325 $$Dose_{LP,j} = \frac{1}{R} C_{RHRW,LP} \frac{1}{n_s} P_{contam} Bt \sum_{i=1}^{n} C_{aer,i} V_{aer,i} DE_i \qquad (7)$$

326

327 $$Dose_{MAC,j} = \frac{1}{R} C_{RHRW,MAC} Bt \sum_{i=1}^{n} C_{aer,i} V_{aer,i} DE_i \qquad (8)$$

Where n_s= number of samples, P_{contam}= binomial probability of contamination with number of samples n_s and probability of contamination p, $C_{aer,i}$ = the concentration of aerosols of diameter i where i =1:10, $V_{aer,i}$ = the volume of each aerosol size bin i calculated as $V = (4/3)\pi r^3$, B = breathing rate (m^3/min), t = exposure duration (min); and DE = alveolar deposition efficiency of size i diameter aerosols.

The aerosol size distributions for aerosols of diameters 1-10 µm from toilet flushing, showering, and hose use are provided by O'Toole et al. (O'Toole et al., 2008b, O'Toole et al., 2009) and are summarized in Table 2. For showering, a conventional showerhead operating at a water temperature of 42°C was chosen. For toilet flushing, a full 9 L flush 420 mm above the toilet was chosen; this category only observed aerosols in one size bin (median 2.5 µm diameter). For gardening, hose use for gardening purposes is assumed to use a "conventional" nozzle on a "spray" setting while car washing would be represented by hosing with a high pressure "water efficient device" on a "jet" setting. Experiments with the largest generation of aerosols in the 1-10 µm diameter range were chosen.

2.3.2. Pool top-up

Partitioning coefficients were used to model swimming in a pool that has been partially filled with RHRW using equations 9 and 10.

$$D_{LP,pool} = \frac{1}{R} C_{RHRW,LP} \frac{1}{134} P_{contam} P_{LP} B t_{pool} D \qquad (9)$$

$$D_{MAC,pool} = \frac{1}{R} C_{RHRW,MAC} P_{MAC} B t_{pool} D \qquad (10)$$

Where P = water to air partitioning coefficient (L per m^3), B = breathing rate (m^3 per min), and t = exposure duration (min). Dilution D was assessed for the same conditions as the ingestion model. The partitioning coefficient for LP in an indoor warm therapy pool was calculated to range from 2.2×10^{-8} to 1.1×10^{-5} L per m^3 in a previous study (Hines et al., 2014). A partitioning coefficient for MAC was calculated in the current study using ratios of median concentrations of nontuberculous mycobacteria in therapy pool water and air reported by Glazer et al., (2007) for sites where NTM was recovered from both air and water ranging from 1.0×10^{-4} to 5.3×10^{-3} L per m^3. The number of minutes of exposure time per swim was derived from a published study (Schets et al., 2011) by combining parameters for males, females, and children using the same method as for swimming

exposure frequency (section 2.2.6). Bathing for hygienic purposes was not considered for inhalation (general population) exposures.

2.3.3. Opportunistic pathogen concentrations in RHRW

The concentrations of LP, *M. avium*, and *M. intracellulare* ranged up to 9.8×10^3 gene copies per L, 1.1×10^5 gene copies per L, and 6.8×10^5 gene copies per L, respectively (Hamilton et al., 2016). *M. intracellulare* is thought to only reside in soil, while *M. avium* or *M. chimaera* are found in water (Wallace et al., 2013). The primer set used in Hamilton et al., 2016 for *M. intracellulare* (Chern et al., 2015) identifies both *M. intracellulare* and *M. chimaera*. As *M. intracellulare* is not expected to be present in water, we assume all *M. intracellulare* enumerated were actually *M. chimaera*. Furthermore, because of the lack of a dose response model specific to *M. chimaera*, we assumed that the dose response relationships in Hamilton et al., 2017 can be applied to the total dose of MAC (*M. avium* and *M. chimaera*). This presents a challenge for adding these on a sample by sample basis where, for example, the concentration of *M. avium* was positive (above the lower limit of detection, LLOD) and above the lower limit of quantification (LLOQ), but the concentration of *M. intracellulare* was positive (above the LLOD) but above the LLOQ. To address this issue, separate interval-censored lognormal distributions were fitted to each dataset (*M. avium* and *M. intracellulare*) using the package fitdistrplus in R, and the simulated distributions were added within the Monte Carlo simulation model to obtain the total MAC count. Therefore, $C_{RHRW,\,MAC} = C_{RHRW,MA} + C_{RHRW,\,MI}$ (Table 1). It is noted that in the original study, there was no significant differences in pathogen concentrations by sampling cluster (Brisbane vs. Currumbin Ecovillage) (Hamilton et al. 2016). The primer sets for *M. avium* and *M. intracellulare* both target the 16S gene, which occurs as a single copy (Chern et al. 2014). Similarly, *L. pneumophila* primer sets targeted the *mip* gene which is a single copy gene (Engleberg et al. 1989). It was therefore assumed that one gene copy was equivalent to one viable, infectious microorganism for *M. avium*, *M. intracellulare*, and LP.

2.4. Dose response

Exponential and Beta-Poisson dose response models (Haas et al. 1999) are stated in equations 11 and 12, respectively, with modifications as described below. Exponential dose response model parameters for LP infection are provided in Table 3 (Armstrong and Haas, 2007). Exponential and Beta-Poisson dose response models for MAC pulmonary infection, disseminated infection, and cervical lymphadenitis were used (Jorgensen et al. 1977 model (1) for cervical lymphadenitis and the

Yangco et al. 1989 model for disseminated infection were chosen and are described in detail in relation to these endpoints elsewhere (Hamilton et al., 2017). Of the three available cervical lymphadenitis dose-response models (Hamilton et al. 2017), the model with a lymph node lesions endpoint (instead of infection) was selected as this was likely to describe a more severe infection representative of cervical lymphadenitis in children. A conversion factor (*C*) for pulmonary infection of 500 is applied to convert the model from the intravenous route to the inhalation route as per Hamilton et al., 2017, by dividing the daily dose by 500 within the inhalation models only. For all other models, *C* = 1.

$$\boldsymbol{P_{inf,daily} = 1 - e^{-rd/C}} \qquad (11)$$

$$\boldsymbol{P_{inf,daily} = 1 - (1 + \frac{d/C}{\beta})^{-\alpha}} \qquad (12)$$

Where $P_{inf,daily}$ = daily probability of infection, *d* = daily dose, and *r*, *α*, and *β* are parameters of the respective dose response models.

For the special case of lymphadenitis in children, disease occurs only in children 1-12 years (Lai et al. 1984) of age and the majority of cases are reported in children 1-5 (Inderlied et al. 1993, Haverkamp et al. 2004, Wolinsky 1995, Lincoln et al, 1972, Hazra et al. 1999, Tebruegge et al. 2016). To account for variability in age groups and difference in susceptibilities among the child population at risk, the daily probability of infection is calculated for children according to equation 13:

$$P_{inf,daily,child,activity} = 1 - \prod_1^i \left(1 - P_{inf,daily,i} P_{attr_i}\right) \qquad (13)$$

Where $P_{inf,daily,child,activity}$ = daily probability of MAC cervical lymphadenitis due to a given ingestion activity (swimming, toilet flushing, etc.) in a child from 1-12 years of age; $P_{inf,daily,i}$ = calculated daily probability of lymphadenitis for children in age bracket *i* where *i* = 1-2, 3-5, or 6-12), P_{attr} = Portion of total age 1-12 MAC-attributable lymphadenitis cases in age bracket *i*. Lai et al. (1984) report that 87% of total diagnosed mycobacterial cervical lymphadenitis cases occurred before age 12. Of the total cases occurring from ages 1-12, 60% occurred between ages 1-3, 27% occurred from ages 3-5, and 13% occurred from ages 5-12; these values are used as $P_{attr,i}$.

For children ages 1-2, the exposure pathways considered are drinking water, eating lettuce, bathing, and swimming in pools topped-up with rainwater. For children ages 3-5, the exposure

pathways considered are drinking, eating lettuce, bathing (instead of showering), swimming in pools topped-up with rainwater, and toilet flushing. For ages 6-12, exposure scenarios considered were considered were drinking, eating lettuce, showering, swimming in pools topped-up with rainwater, and toilet flushing.

2.5. Risk characterization

Annual risk was calculated as per Equation 14.

$$\boldsymbol{P_{inf,ann} = 1 - \prod_1^{nf_j}(1 - P_{inf,daily})} \quad (14)$$

Where *n* is the yearly frequency and *f* is the daily frequency of the activity *j*. Frequency f_j = 1 unless otherwise stated in Table 2. A sensitivity analysis was conducted to identify variables contributing to uncertainty using 100,000 Monte Carlo iterations. All computations were performed in R (www.rproject.org) and using the mc2d package (Pouillot et al., 2010). Random sampling of daily risks with replacement was conducted as per the preferred method for annualizing probability of infection using 100,000 iterations (Karavarsamis and Hamilton, 2010).

In addition to annual risks for each exposure scenario, total annual infection risks for each population were calculated according to equation 15. A similar approach has been used to pool risks from multiple pathogens by previous QMRA studies (de Man et al. 2014b, Soller et al. 2016, Ahmed et al. 2010).

$$P_{inf,ann,total,s,e,p,c} = 1 - \prod_1^j (1 - X_{a_j} P_{inf,ann,a_j}) \quad (15)$$

Where $P_{inf,ann,total,s,e,p,l}$ = the total annual risk incurred from each scenario *s* where *s* = inhalation or ingestion annual risk from *j* activities a_1, a_2, a_3..a_j where a_j = showering, drinking, etc., and $X_{a,j}$ = portion of sampling cluster location *c* that uses rainwater for each activity (Supplemental Table 1). For child bathing in the ingestion scenario, because exposure volumes were not available, it was assumed that the same volume is ingested during indoor bathing for hygienic purposes in a bathtub as during swimming in an outdoor pool (Schets et al. 2011). Total risk is calculated for health endpoint *e* = pulmonary infection, cervical lymphadenitis, or disseminated infection and *p* = healthy

populations, children, or vulnerable/immune-compromised populations that are relevant for each of the previous endpoints, respectively. Total risks for each scenario are specific to each rainwater-using population group *p,* and are therefore not extrapolated to a general population level that includes non-rainwater users. For the pulmonary infection endpoint, LP and MAC risks are included in the same equation to compute total pulmonary disease risks, assuming the infection risks from these organisms are additive and that infection with one pathogen would not preclude infection with the other.

The Spearman rank correlation coefficient was used to identify the most important predictive factors of annual infection or clinical severity infection risk, were 0 is no influence and -1 or +1 when the output is wholly dependent on that input. The model inputs were ranked based on their correlation coefficient with the output variable, annual risk.

3. Results

3.1 Ingestion

Annual risks for each ingestion scenario are shown in Fig. 2 and compared to a hypothetical 1×10^{-4} drinking water annual infection risk benchmark (Regli et al., 1991). Consumption of rainwater presented the highest risk for both cervical lymphadenitis in children and disseminated infection for immune-compromised adults. The use of drinking water POU filters reduced total risks by approximately 90% (<1 log), but did not bring drinking water risks below 10^{-4}. For children, bathing (instead of showering) in untreated, undiluted rainwater also resulted in high annual risks above 10^{-4}. In all drinking water scenarios, the median annual risk was $> 1 \times 10^{-4}$. For child non-recreational exposures, consumption of lettuce irrigated with RHRW, showering, and toilet flushing had median annual lymphadenitis risks below the benchmark, however the 95$^{\%}$ confidence limit for toilet flushing exceeded the benchmark. For immune-compromised non-recreational scenarios, only car washing and clothes- washing had median annual disseminated infection risks below the benchmark and the 95% confidence limit for car washing exceeded this value. There is no risk benchmark for swimming in pools, although there are recreational standards for microbiological risks associated with freshwater and marine beaches of 8 and 19 cases of highly credible gastrointestinal illness (HCGI) per 1,000 recreators in fresh and marine waters, respectively (US EPA, 2011b). Eight cases of HCGI per 1,000 is equivalent to 36 cases of gastrointestinal illness per 1,000 recreators based on a more encompassing definition of gastrointestinal illness used in the USEPA National Epidemiological and

475 Environmental assessment of Recreational Water (NEEAR) studies (USEPA, 2011b). These
476 definitions are not particularly compatible with the health endpoints used in this analysis, and are
477 therefore not used for comparison here. Generally, swimming pool risks were higher for
478 vulnerable/immune-deficient populations than for children. For child exposures, swimming pool water
479 would need to be diluted to 50% RHRW: 50% sterile water in order to have a median risk below or
480 equal to 1×10^{-4} for children swimming at an average rate (n= 32 swims per year) and 10% RHRW:
481 sterile water for children with an upper-bound exposure based on an upper bound 95th percentile
482 estimate of 122 swims per year. Of the dilution scenarios tested (10%, 50%, and 90% RHRW: sterile
483 water), the median annual pool risks for immune-compromised individuals were never below 10^{-4}. If
484 using a less stringent recreational water-based risk comparison point of approximately 10^{-2} on an
485 annual basis, a dilution of 10% RHRW: sterile water would be needed to achieve median annual risks
486 below this level for disseminated infection. All child median annual risks were below this level.
487 A sensitivity analysis for ingestion risk scenarios is shown in Table 6. The most influential
488 predictor of variability in annual risk for all scenarios was the concentration of MAC in RHRW ($C_{MA,}$
489 C_{MI}) (Spearman rank correlation coefficients ranging from 0.16-0.19 and 0.70- 0.90 for MA and MI,
490 respectively). However, as C_{RHRW} was calculated by adding the concentration of *M. avium* and *M.*
491 *intracellulare* in RHRW, the sensitivity analysis determined that the concentration of *M. intracellulare*
492 was more influential as this is more commonly present in RHRW and in higher concentrations than *M.*
493 *avium* on average. The dose response parameter *r* and volume of water ingested (V_{ing}) or retained by
494 lettuce (V_R) were the second and third most influential parameters, respectively.
495 *3.2 Inhalation*
496 Annual risks for each inhalation scenario are shown in Fig. 3. The median annual LP risks for
497 showering, and garden hosing exceeded a 1×10^{-4} benchmark. Risks for toilet flushing and car
498 washing were below the benchmark. Risks were highest for showering and pool top-up. For MAC, all
499 95% confidence intervals were below the benchmark value. Annual pool swimming risks were above
500 10^{-4} but below 10^{-2}.
501 A sensitivity analysis for inhalation risk scenarios is shown in Table 6. The most influential
502 predictor of variability in daily risk for toilet flushing, showering, garden hosing, or car washing was the
503 concentration of LP and/or MAC in RHRW ($C_{RHRW,}$ $C_{MI,}$ or C_{MA}) (Spearman rank correlation coefficients
504 ranging from 0.14- 0.88). For LP toilet flushing scenario only, the concentration of aerosols (C_{aer}) was

505 the most important predictor (ρ = 0.80) while for MAC toilet flushing, C_{aer} was the second most
506 important predictor (ρ = 0.58). For LP scenarios, the probability of contamination (P_{contam}) was also an
507 important factor (Spearman rank correlation coefficients ranging from 0.26 to 0.47). For all pool
508 scenarios, the partitioning coefficient (*P*) was either the first or second-most important predictor
509 (Spearman rank correlation coefficients ranging from 0.29 to 0.50).

510 *3.3 Total annual risks*

511 In order to quantify the risks for each relevant population/ exposure route, a distribution of total annual
512 risks was computed for each scenario. For cervical lymphadenitis in children and disseminated
513 infection in immune-compromised populations via a combination of all oral ingestion exposure routes
514 (Fig. 4), risks for immune-compromised populations were higher than for children, and higher for the
515 Currumbin Ecovillage study population than the Brisbane study population based on survey
516 responses. Total annual risks were not substantially impacted by the dilution of RHRW used in pools
517 with sterile water or the number of swims per year The use of drinking water POU filters reduced total
518 risks by approximately 90% (<1 log). All median total risks were above a 1×10^{-4} benchmark.

519 Total inhalation risks from all aerosol-generating activities reported in the survey for each
520 sampling cluster are summarized in Fig. 5. Median total annual LP pulmonary infection risks were
521 approximately 4-5 orders of magnitude higher than MAC pulmonary infection risks for all scenarios,
522 and all 95% confidence intervals for LP total risks were above the 1×10^{-4} benchmark while all those
523 for MAC were below this value. Therefore, total pulmonary infection risks (LP + MAC) were driven by
524 LP risks rather than MAC risks. Total annual risks for LP were slightly higher for the Brisbane
525 compared to the Currumbin Ecovillage, while total annual MAC risks were lower for Currumbin than
526 for Brisbane. The number of swims per year and pool dilution did not have a substantial impact on
527 differences in risk between scenarios.

528

529 ## 4. Discussion

530 The current study is the first investigation to assess MAC risks for human-relevant, non-MAP
531 species of MAC in an aquatic environment. For MAC, the drinking water route of exposure presents
532 the greatest risks for susceptible populations including children and the immune-compromised. For
533 these populations, the simulated risks do not support the conclusion that it would be appropriate to
534 drink rainwater or use it for bathing children without treatment. The use of a filter reduced risks but did

not reduce them to below benchmark levels; additionally, opportunistic pathogens such as MAC can colonize POU filtration systems (Rodgers et al., 1999), limiting their utility for mitigating drinking water risks in these circumstances.

For swimming pool use, risks can be lowered through dilution with treated water. Although there is no risk benchmark for opportunistic pathogens in pools, this work indicates that a dilution to 90% rainwater would bring median risks below a recreational benchmark. However, the risk simulations conducted do not indicate that it would be feasible to reduce annual risks for immune-compromised populations to below 10^{-4} but for children, a median annual risk would be below this level for 50% dilution RHRW: sterile water. A 10^{-4} risk level may be more appropriate for comparison in order to reduce risks in this case. However, this dilution factor for children can be lessened depending on the recreational water quality criteria used for comparison. *Legionella pneumophila* risks were above the drinking water benchmark for all scenarios except toilet flushing and car washing, and were greater than MAC risks in all cases. Showering and swimming pool use presented the highest risks for pulmonary infection.

Both inhalation and ingestion simulations indicate that for non- immune-compromised populations, toilet flushing is one of the lowest-risk scenarios and is likely to be an appropriate use for rainwater. Similarly, clothes washing and car washing represented the two other lower-risk scenarios. Clothes washing, garden hose use, and car washing scenarios were not considered for children; however some children may perform these types of chores, although it is likely to be a small percentage of children and there is likely to be considerable variability in these behaviors (Klein et al. 2009). For children, consumption of lettuce was also below the benchmark risk. All individual MAC pulmonary infection inhalation risks for healthy populations were below the drinking water benchmark. This could be affected by the fact that a conversion factor of 500 was used to convert the intravenous to inhalation dose response route (Hamilton et al., 2017). Due to the lack of dose response models for pulmonary infections in susceptible populations, further investigation of potential dose response models for this purpose is recommended. When considering “total” risks for populations performing all the possible activities using rainwater, the risk simulations indicated that total risks could be high and in no case was below 10^{-4}. This supports that using RHRW as a sole source of water could incur potential risks and additional assessment of combinations of activity patterns yielding risks within certain ranges would be beneficial for decision-making purposes.

565 An important factor in this work was the regional preference for various uses of RHRW. When
566 examining differences between two sampling clusters in Brisbane and the Currumbin Ecovillage,
567 ingestion risks and inhalation risks for total inhalation pathogen load (LP and MAC) were higher for
568 the Currumbin Ecovillage compared to Brisbane. This is likely due to the higher degree of RHRW
569 application for potable uses in Currumbin compared to Brisbane. When examining total inhalation
570 risks from all uses (showering, garden hose, car washing, etc.) and considering *L. pneumophila* and
571 MAC risks separately, Currumbin risks for LP were higher than Brisbane but MAC risks were lower
572 than Brisbane. This may be due to higher RHRW use for pools and showers in Brisbane than
573 Currumbin. MAC pool inhalation risks were computed using a partitioning coefficient, which is
574 reported to be higher for MAC than for LP. *L. pneumophila* shower risks were the highest compared to
575 other scenarios, indicating that showering may have had a greater influence on total annual
576 pulmonary risk for Currumbin compared to the other scenarios.
577 For previous studies focusing on LP risks in RHRW with LP concentrations of 60 to 170 gene
578 copies (assumed equivalent to cells) per L rainwater, between 3.0×10^{-2} and 8.6×10^{-2} LP infections
579 per 10,000 exposed people per shower exposure and 1.8×10^{-2} - 5.1×10^{-2} infections per 10,000
580 exposed people per hosing exposure were expected (Ahmed et al., 2010). This would be between an
581 annual probability of infection of approximately 1.09×10^{-3} and 3.13×10^{-3} and is comparable with the
582 risks calculated in the current study. A case of Legionnaires disease has also been previously linked
583 to the usage of a garden hose (Piso et al., 2007).
584 Another study of rainwater used as a source of water for splash parks estimated a mean
585 *Legionella* infection risk of 9.3×10^{-5} for a 3.5 minute splash park exposure for children and 1.1×10^{-4}
586 for adults, but that for a 2 h exposure this risk would be approximately 2.8×10^{-3} (de Man et al.,
587 2014a). Although the pool exposure model is different than that used in the current study, de Man et
588 al. work supports that pool risks can be potentially high compared to other exposure routes. The
589 current QMRA did not consider ingestion of mouthfuls of water as in de Man et al. models due to the
590 difference between active splash parks and private pools. However, exposures to pool water could
591 potentially be higher in some circumstances if mouthfuls of water are considered. Private household
592 pool or whirlpool use has indeed been linked to Legionnaires disease cases (Euser et al., 2010). It is
593 noted in the current QMRA that although dilution of RHRW with sterile water was considered, RHRW
594 users may dilute rainwater with treated tap-water which may not be free of LP and MAC but is likely to

595 contain fewer pathogens than RHRW (Whiley et al., 2014). Additionally, some chlorine residual
596 present in municipal drinking water could contribute to pathogen removal. However, LP (Cooper and
597 Hanlon, 2010), and especially MAC (Falkinham 3rd, 2003; Steed and Falkinham 3rd, 2006; Taylor et
598 al., 2000) are resistant to disinfection including chlorination, and would not be likely to decline
599 significantly at low levels of chlorine residual. Chlorine treatment in pools by owners could contribute
600 to pathogen removal and could be considered in a more detailed assessment of opportunistic
601 pathogens in pools.
602 Similarly to the previous QMRAs for RHRW (Ahmed et al., 2010; de Man et al., 2014a), in the
603 absence of information regarding the relationship between total gene copies and the viability of
604 pathogens in rainwater tanks, one gene copy was considered equivalent to one viable, infectious
605 pathogen. This assumption could potentially lead to an overestimation of risks; however, culture-
606 based assessments of pathogens can neglect to quantify viable but non-culturable (VBNC)
607 microorganisms, and therefore underestimate risks. If a 1% or 10% viability assumption is made, total
608 annual risks would be 1 to 2 orders of magnitude lower, respectively.
609 Finally, regarding the clothes washing scenario, MAC can be present in fecal material (Yajko et
610 al., 1993) that can be introduced to the laundry cycle through soiled clothing and provide additional
611 pathogen loading. A more in-depth QMRA model for MAC risks due to clothes washing might
612 consider transfer of fecal-associated MAC to water and hand-to-mouth contact after clothes
613 laundering and impact of any disinfectants used (Callewaert et al., 2015; Gerba and Kennedy, 2007,
614 Gibson et al., 1999; Lopez et al., 2013).
615 Regarding the management of RHRW risks, toilet lids can be closed prior to flushing to prevent
616 the spread of mists. Similarly, less aerosol-efficient showerheads can be installed in showers for
617 which rainwater is used as a source. For pool-users, pool filters can be installed and rainwater can be
618 diluted with treated water in order to potentially lower risks. In this study, only under-sink POU filters
619 were considered for mitigating drinking water risks, however, other methods such as solar-disinfection
620 have been explored for use with rainwater tanks which could provide higher log removals (Reyneke et
621 al., 2016). Treatment systems designed to treat all water entering the home from a rainwater tank as
622 opposed to only water obtained via a kitchen sink tap would be beneficial for reducing risks. More
623 exploration of *in situ* field performance of various rainwater treatment systems over time is warranted
624 as a means for approaching pathogen risk mitigation for RHRW.

5. Conclusions

- Regional use preferences for roof-harvested rainwater had an impact on the rank order of ingestion and inhalation risks for opportunistic pathogens
- Based on the results of the *Mycobacterium avium* quantitative microbial risk assessment ingestion scenarios, harvested rainwater is not recommended for drinking in child or immune-compromised populations
- For healthy populations, showering and garden hosing are not recommended for harvested rainwater
- Compared to *Mycobacterium avium* infection risks, potential *Legionella* risks should drive risk mitigation strategies for the inhalation route of exposure

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927 **Table 1**

928 Monte Carlo simulation input parameters for ingestion scenarios (for MAC only)

Parameter	Symbol	Unit	Value	Distribution	Source
Child population parameters					
Portion of total age 1-12 MAC-attributable lymphadenitis cases ages 1-2	$P_{attr,i}$	Proportion	0.60	Point	(Lai et al. 1984)
Portion of total age 1-12 MAC-attributable lymphadenitis cases ages 3-5	$P_{attr,i}$	Proportion	0.27	Point	(Lai et al. 1984)
Portion of total age 1-12 MAC-attributable lymphadenitis cases ages 6-12	$P_{attr,i}$	Proportion	0.13	Point	(Lai et al. 1984)
Drinking water					
Intake rate (general population)	$V_{ing,dw}$	L per day	μ = -0.529, σ = 0.882	Lognormal[a]	(USEPA 2011a)
Intake rate (children 1-2 years)	$V_{ing,dw}$	L per day	μ = -1.578, σ = 0.824	Lognormal[a]	(USEPA 2011a)
Intake rate (children 3-5 years)	$V_{ing,dw}$	L per day	μ = -1.457 , σ = 0.823	Lognormal[a]	(USEPA 2011a)
Intake rate (children 6-12 years)	$V_{ing,dw}$	L per day	μ = -1.284, σ = 0.897	Lognormal[a]	(USEPA 2011a)
Exposure frequency	n_{dw}	-	365	Point	Assumption
Log removals due to filtration	L	logs	Min=0.4, Max=2	Uniform	(Jordan 2008)
Toilet flushing					
Volume ingested	$V_{ing,t}$	mL	Min = 0.01, Max = 0.3	Uniform	(NRMMC-EPHC-AHMC 2006, Schoen et al. 2014)
Toilet flushes per day	n_t	Flushes per day	5	Point	(Mayer and DeOreo 1999)
Showering					
Volume ingested	$V_{ing,sh}$	mL	Min = 0.058, Max = 1.9	Uniform	(Ahmed et al. 2010)
Showers per year	n_{sh}	Number per year	365	Point	Assumption
Garden hosing					
Volume ingested	$V_{ing,gh}$	mL	Min = 2×10^{-9}, Max = 1.1×10^{-3}	Uniform	(Ahmed et al. 2010, NRMMC-EPHC-AHMC 2006, Schoen et al. 2014)
Hosing events per year	n_{gh}	Number per year	90	Point	(NRMMC-EPHC-AHMC 2006)
Car washing					
Volume ingested	$V_{ing,cw}$	mL	Min = 0.06, Max = 3.79	Uniform	(Sinclair et al. 2016)
Car washing events per year	n_{cw}	Number per year	12	Point	(Villarreal and Dixon 2005)
Pool top-up and bathing					
Dilution Factor (RHRW: sterile water)	D	%	10 50 90 100 (bathing only)	Point	Assumption

Volume ingested per swim (general population)	$V_{ing,sw}$	mL	μ = 2.92 , σ = 1.43	Lognormal	(Dufour et al. 2006, Schoen et al. 2014)
Volume ingested per swim (children age 1-12) or bath (children age <6)	$V_{ing,sw}$	mL	r = 0.81, λ = 63	Gamma	(Schets et al. 2011)
Swims per year (general population)	n_{sw}	Number per year	Average 21 Upper bound 72	Point	(Schets et al. 2011)[b]
Swims per year (children age 1-12)	n_{sw}	Number per year	Average 32 Upper bound 122	Point	(Schets et al. 2011)[b]
Baths per year	n_{bath}	Number per year	365	Point	Assumption
Clothes washing					
Volume ingested	$V_{ing,cw}$	mL	0.01	Point	(NRMMC-EPHC-AHMC 2006)
Number of times clothes are washed with rainwater per year	n_{cw}	Number per year	100	Point	(NRMMC-EPHC-AHMC 2006)
Produce consumption					
Volume irrigated water retained on lettuce	V_R	mL per g	μ = 0.108 , σ = 0.019	Normal, truncated at 0	(Shuval et al. 1997)
Internalized fraction of MAC in irrigation water	F_{int}	Proportion	Min=1.13×10^{-5}, Max=9.49×10^{-4}	Uniform	(Kaevska et al. 2014)
In-field decay on surface of plant	$K_{f,s}$	d-1	-0.0484	Point	(Cook et al. 2013)
Log reductions due to lettuce washing	W	Logs	μ = 0.3, σ = 0.1	Normal, truncated at 0	(Holvoet et al. 2014)
Time in field between irrigation and harvest	t_f	day	2	Point	(Barker et al. 2013)
Intake rate (general population)	I	g/kg-day	μ = -2.30, σ = 1.64	Lognormal	(Barker et al. 2013)
Intake rate (1-2 years)	I	g/kg-day	μ = -3.388, σ = 1.727	Lognormal	(USEPA 2011a)
Intake rate (3-5 years)	I	g/kg-day	μ = -2.905, σ =1.694	Lognormal	(USEPA 2011a)
Intake rate (6-12 years)	I	g/kg-day	μ = -2.860, σ = 1.475	Lognormal	(USEPA 2011a)
Body weight (general)	B_{gen}	kg	μ = 4.136 , σ = 0.434	Lognormal	Modelled parameters based on (ABS 1998, ABS 2016, CSIRO 2008)
Body weight (1-2 years)	B_{1-2}	kg	μ = 2.522 , σ = 0.152	Lognormal	(USEPA 2011a)
Body weight (3-5 years)	B_{3-5}	kg	μ = 2.902, σ = 0.206	Lognormal	(USEPA 2011a)
Body weight (6-12 years)	B_{6-12}	kg	μ = 3.407 , σ = 0.323	Lognormal	(USEPA 2011a)
Proportion who consume lettuce (general population)	F_{cl}	Proportion	0.53	Point	(USEPA 2011a)
Proportion who consume lettuce (1-2 years)	F_{cl}	Proportion	0.21	Point	(USEPA 2011a)
Proportion who consume lettuce (3-5 years)	F_{cl}	Proportion	0.29	Point	(USEPA 2011a)
Proportion who consume lettuce (6-12 years)	F_{cl}	Proportion	0.37	Point	(USEPA 2011a)
Lettuce consumption events per year	n	Days per year	365	Point	USEPA intake rates reported as normalized per day

[a]Lognormal parameters mean, standard deviation (μ, δ) calculated from population (normal) parameters ($\bar{x}$, s) using standard formulae as follows: $\mu = \ln(\bar{x}^2/(s^2+\bar{x}^2)^{1/2})$, $\delta = [\ln(1+(s^2/\bar{x}^2))]^{1/2}$, where $\bar{x}$ is the sample mean and s^2 is the sample standard deviation. [b] Estimates from Schets et al. modified based on a 9-month Queensland swim season.

949 **Table 2**.

950 Monte Carlo simulation input parameters for inhalation scenarios.

Parameter	Symbol	Unit	Value	Distribution	Source
Breathing rate, light activity, breathing cycle period 8 s and 1 L tidal volume	B	m^3 per min	0.013-0.017	Uniform	(USEPA 2011a)
Deposition efficiency (diameter)	DE_i	Proportion		Uniform	(Heyder et al. 1986)
1			Min = 0.23, Max =0.25		
2			Min = 0.4, Max =0.53		
3			Min = 0.36, Max =0.62		
4			Min = 0.29, Max =0.61		
5			Min = 0.19, Max =0.52		
6			Min = 0.1, Max =0.4		
7			Min = 0.06, Max =0.29		
8			Min = 0.03, Max =0.19		
9			Min = 0.01, Max =0.12		
10			Min = 0.01, Max =0.06		
Toilet flushing					
Toilet flushes per day	f_t	Flushes per day	5	Point	(Mayer and DeOreo 1999)
Time in bathroom after flush	t_t	Min per flush	Min = 1, Max = 5	Uniform	Lim et al. (2015)
Concentration of aerosols of diameter *i*:	$C_{aer,i}$	# aerosols per cm^3 of air	μ = -1.246, σ = 1.885	Lognormal[a]	(O'Toole et al. 2009)
2.5					
Showering					
Shower duration	t_{sh}	min per day	15	Point	(Schoen and Ashbolt 2011)
Showers per year	n_{sh}	Showers per year	365	Point	Assumption
Concentration of aerosols of diameter *i*:	$C_{aer,i}$	# aerosols per cm^3 of air		Lognormal	(O'Toole et al. 2009)
1.5					
2.5			μ = 3.718, σ = 0.296		
4.5			μ = 3.699, σ = 0.170		
8			μ = 5.549, σ = 0.348		
			μ = 6.185, σ = 0.309		
Garden hosing					
Hosing duration	t_{gh}	min	7	Point	(Ahmed et al. 2010)
Hosing events per year	n_{gh}	Number per year	90	Point	(NRMMC-EPHC-AHMC 2006)
Concentration of aerosols of diameter *i*:	$C_{aer,i}$	# aerosols per cm^3 of air		Lognormal	(O'Toole et al. 2009)
1.5			μ = 5.728, σ = 0.274		
2.5			μ = 4.949, σ = 0.333		
4.5			μ = 3.047, σ = 0.586		
8			μ = -2.451=, σ=1.579		
Car washing					

Car washing duration	t_{cw}	min	10	Point	(O'Toole et al. 2008b)
Car washing events per year	n_{cw}	Number per year	12	Point	(Villarreal and Dixon 2005)
Concentration of aerosols of diameter *i*:	$C_{aer,i}$	# aerosols per cm^3 of air		Lognormal	(O'Toole et al. 2009)
1.5			μ = 6.187, σ = 0.476		
2.5			μ = 4.665, σ = 0.420		
4.5			μ = 1.742, σ = 0.591		
8			μ = -1.551, σ = 0.833		
Pool top-up					
Partitioning coefficient- *L. pneumophila*	P_{LP}	L per m^3	Min = 2.2×10^{-8}, Max = 1.1×10^{-5}	Uniform	(Hines et al. 2014)
Partitioning coefficient- MAC	P_{MAC}	L per m^3	Min = 1.0×10^{-4}, Max = 5.3×10^{-3}	Uniform	(Glazer et al. 2007)
Dilution Factor (RHRW:sterile water)	*D*	%	10	Point	Assumption
			50		
			90		
Pool exposure time per swim (general)	t_{pool}	min	μ = 4.082, σ = 0.564	Lognormal	(Schets et al. 2011)
Swims per year (general population)	n_{sw}	Number per year	Average 21	Point	(Schets et al. 2011)[b]
			Upper bound 72		

[a] Lognormal parameters mean, standard deviation (μ, δ) calculated from population (normal) parameters ($\bar{x}$, s) using standard formulae as follows: $\mu = \ln(\bar{x}^2/(s^2+\bar{x}^2)^{1/2})$, $\delta = [\ln(1+(s^2/\bar{x}^2))]^{1/2}$, where $\bar{x}$ is the sample mean and s^2 is the sample standard deviation. [b] Estimates from Schets et al. modified based on a 9-month Queensland swim season.

966 **Table 3.**

967

968 Monte Carlo simulation dose response input parameters.

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Parameter	Symbol	Unit	Value	Distribution	Source
L. pneumophila					
Dose response parameter for *L. pneumophila*, infection endpoint	*r*	Unitless	μ = -2.934, σ = 0.488	Lognormal[a]	(Armstrong and Haas 2007)
MAC					
Dose response parameter for M. avium- pulmonary infection (subclinical infection endpoint)	*r*	Unitless	μ = -13.742, σ = 0.208	Lognormal	(Hamilton et al. 2017)
Conversion factor from intravenous to inhalation route for pulmonary infection model	*C*	Unitless	500	Point	(Hamilton et al. 2017)
Dose response parameters for disseminated infection	*α* *β*	Unitless	0.201 1.15×10^{-6}	Point	(Hamilton et al. 2017)
Dose response models for cervical lymphadenitis in children	*r*	Unitless	μ = -19.006, σ = 1.008	Lognormal	(Hamilton et al. 2017)

970 [a] Lognormal parameters mean, standard deviation (μ, δ) calculated from population (normal) parameters ($\bar{x}$, s) using standard formulae as
971 follows: $\mu = \ln(\bar{x}^2/(s^2+\bar{x}^2)^{1/2})$, $\delta = [\ln(1+(s^2/\bar{x}^2))]^{1/2}$, where $\bar{x}$ is the sample mean and s^2 is the sample standard deviation.

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Table 4.

Monte carlo simulation input parameters for pathogen concentrations.

CONCENTRATIONS IN RHRW					
M. avium	$C_{RHRW,MA}$	# per L	μ = 0.723, σ = 4.349	Lognormal[a]	(Hamilton et al. 2016)
M. intracellulare	$C_{RHRW,MI}$	# per L	μ = 6.720, σ = 2.410	Lognormal	(Hamilton et al. 2016)
L. pneumophila, positive samples only	$C_{RHRW,LP}$	# per L	μ = 8.080, μ = 0.745	Lognormal	(Hamilton et al. 2016)
Probability of *L. pneumophila* occurrence	P_{contam}	Fraction	n = 134, p = 0.03	Binomial	(Hamilton et al. 2016)
Recovery efficiency	*R*	Fraction	0.84	Point	(Hamilton et al. 2016)

[a] Lognormal parameters mean, standard deviation (μ, δ) calculated from population (normal) parameters ($\bar{x}$, s) using standard formulae as follows: $\mu = \ln(\bar{x}^2/(s^2+\bar{x}^2)^{1/2})$, $\delta = [\ln(1+(s^2/\bar{x}^2))]^{1/2}$, where $\bar{x}$ is the sample mean and s^2 is the sample standard deviation.

Table 5

Sensitivity analysis with Spearman rank correlation coefficients for ingestion risks. Risks for endpoints of children with cervical lymphadenitis and severe immune deficiency with disseminated infection are shown.

Scenario	Population	Parameter (Spearman rank correlation coefficient)								
		B	C_{MA}	C_{MI}	F_{int}	*I*	*L*	*r*	V_{ing}	*W*
Drinking water- filtration	Children		0.16	0.73				0.34	0.15 (1 to 2) 0.08 (3 to 5) 0.06 (6 to 12)	
	Immune-comp.		0.17	0.76			-0.36		0.30	
Drinking water- no filtration	Children		0.18	0.80				0.37	0.16 (1 to 2) 0.08 (3 to 5) 0.05 (6 to 12)	
	Immune-comp.		0.18	0.83					0.33	
Lettuce	Children	0.02 (1 to 2) 0.03 (3 to 5) 0.03 (6 to 12)	0.16	0.74	-0.01	0.20 (1 to 2) 0.20 (3 to 5) 0.15 (6 to 12)		0.34	0.05*	-0.08
	Immune-comp.	0.14	0.16	0.71	0.002	0.54			0.05*	-0.08
Shower	Children (6-12 y)		0.17	0.78				0.36	0.26	
	Immune-comp.		0.18	0.85					0.29	
Bathing	Children (1-5 y)		0.16	0.71				0.33	0.46	
Hose	Immune-comp.		0.18	0.82					0.33	
Car	Immune-comp.		0.19	0.84					0.31	
Toilet	Children (>3 y)		0.17	0.78				0.37	0.27	
	Immune-comp.		0.19	0.85					0.29	
Clothes washing	Immune-comp.		0.19	0.90						
Pool, D = 10%, n = Avg.	Children		0.16	0.71				0.33	0.46	
	Immune-comp.		0.17	0.76					0.51	
Pool, D = 50%, n = Avg.	Children		0.16	0.71				0.33	0.46	
	Immune-comp.		0.17	0.75					0.50	
Pool, D = 90%, n = Avg.	Children		0.16	0.71				0.33	0.46	
	Immune-comp.		0.17	0.75					0.50	
Pool, D = 10%, n = High	Children		0.15	0.70				0.33	0.46	

	Immune-comp.	0.16	0.75		0.50
Pool, n = 50%, n = High	Children	0.16	0.71	0.33	0.45
	Immune-comp.	0.16	0.76		0.50
Pool, D = 90%, n = High	Children	0.16	0.71	0.33	0.46
	Immune-comp.	0.17	0.75		0.50

*For the lettuce scenario, the coefficient for volume of water retained by lettuce (V_R) is shown

Table 6

Sensitivity analysis with Spearman rank correlation coefficients for inhalation risk scenarios. Risks for endpoints of pulmonary infection in healthy populations with *L. pneumophila* or MAC are shown.

Scenario	Population	Parameter (Spearman rank correlation coefficient)														
		B	$C_{aer1.5}$	$C_{aer2.5}$	$C_{aer4.5}$	C_{aer8}	C_{RHRW}	C_{MA}	C_{MI}	$DE_{1.5}$	$DE_{2.5}$	$DE_{4.5}$	DE_{8}	*P*	P_{contam}	*r*	*t*
Toilet flushing	LP	0.04		0.80			0.31				0.05				0.26	0.20	0.18
	MAC	0.03		0.58				0.14	0.65		0.04					0.06	0.13
Shower	LP	0.06	0.002	0.003	0.10	0.16	0.59			0.002	-0.002	0.05	0.19		0.46	0.39	
	MAC	0.03	-0.002	-0.005	0.05	0.07		0.19	0.88	0.005	-0.005	0.03	0.09			0.09	
Garden hose	LP	0.06	0.03	0.13	0.16	0.003	0.60			0.03	0.05	0.05	-0.002		0.47	0.39	
	MAC	0.03	0.02	0.07	0.08	-0.001		0.19	0.88	0.01	0.02	0.03	-0.005			0.08	
Car wash	LP	0.06	0.12	0.15	0.06	0.07	0.61			0.07	0.04	0.01	0.03		0.46	0.39	
	MAC	0.04	0.06	0.08	0.03	0.04		0.19	0.88	0.03	0.02	0.02	0.02			0.08	
Pool, D=10%, n=Avg.	LP	0.05					0.45							0.50	0.36	0.30	0.34
	MAC	0.04						0.19	0.82					0.29		0.08	0.20
Pool, D=50%, n=Avg.	LP	0.05					0.46							0.50	0.36	0.29	0.34
	MAC	0.03						0.18	0.81					0.29		0.08	0.21
Pool, D=90%, n=Avg.	LP	0.04					0.45							0.50	0.37	0.30	0.34
	MAC	0.03						0.18	0.81					0.29		0.08	0.21
Pool, D=10%, n=High	LP	0.05					0.45							0.50	0.36	0.29	0.34
	MAC	0.03						0.18	0.82					0.30		0.08	0.21
Pool, D=50%, n=High	LP	0.05					0.45							0.50	0.36	0.30	0.34
	MAC	0.02						0.18	0.82					0.29		0.08	0.21
Pool, D=90%, n=High	LP	0.05					0.45							0.50	0.36	0.30	0.34
	MAC	0.03						0.17	0.81					0.30		0.07	0.21

1019 Fig1

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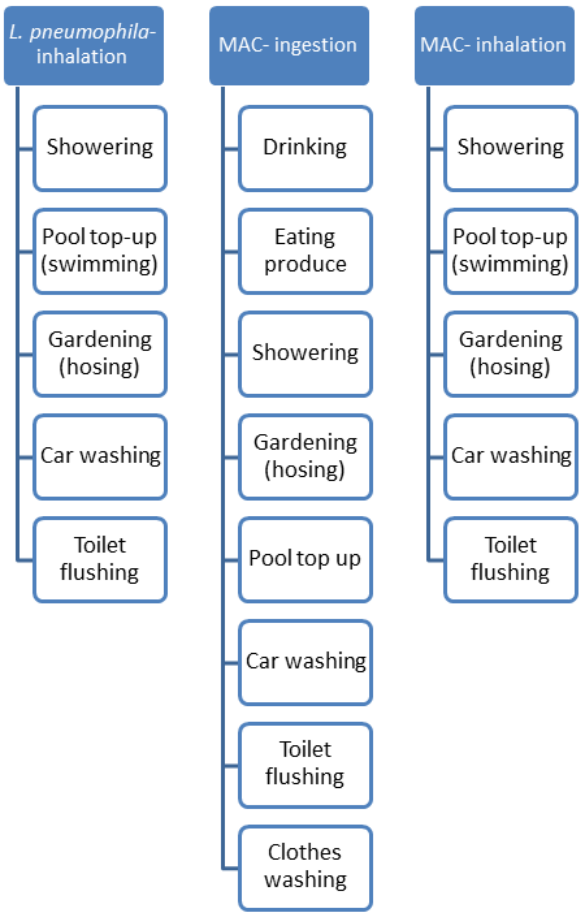

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1025 **Fig. 1.** Exposure routes for *L. pneumophila* and MAC in roof-harvested rainwater

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1029 Fig2

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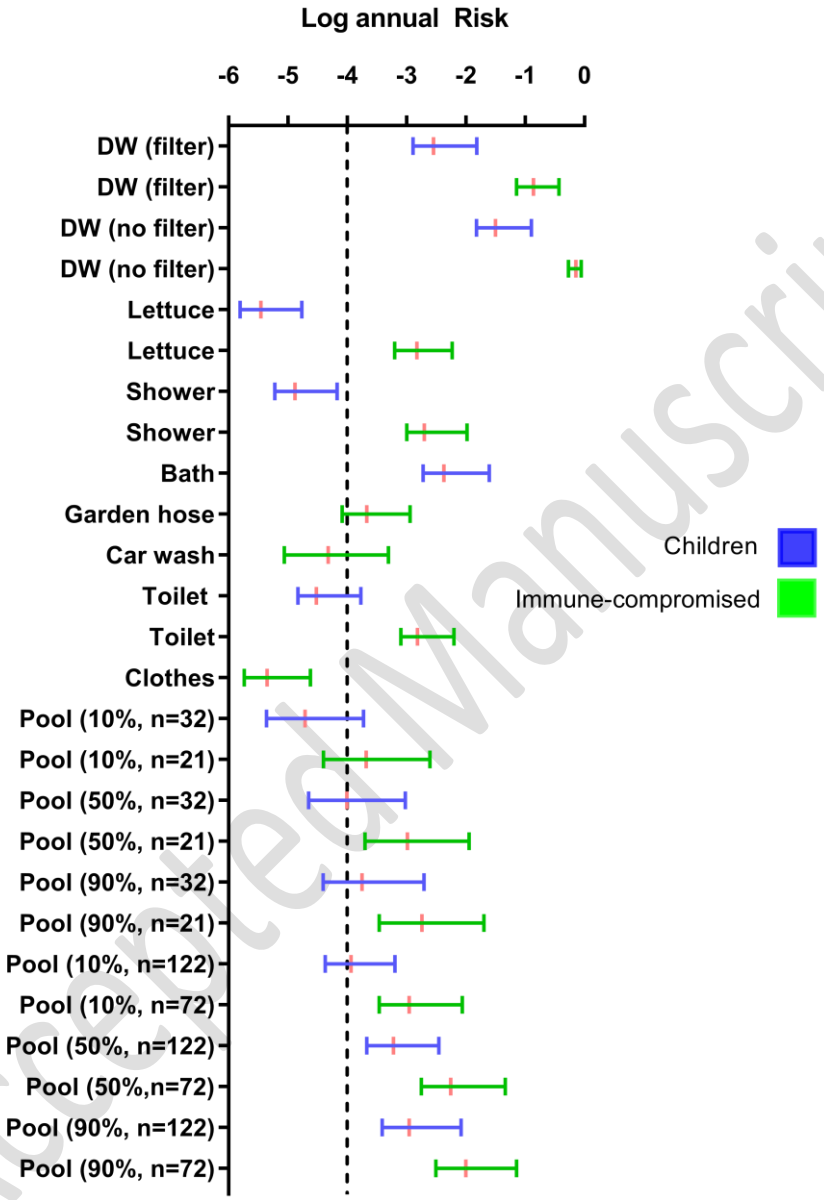

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1033 **Fig. 2**. Annual cervical lymphadenitis (children) or disseminated infection (immune-compromised
1034 populations) risks for ingestion of *Mycobacterium avium* complex through food or water exposure
1035 scenarios. Median and 95% confidence intervals and scenario analysis for various pool dilution levels (D
1036 = 10%, 50%, or 90% RHRW: sterile water), with or without drinking water under-sink point of use filtration
1037 (filtration/no filtration), and swimming frequency assumptions (32 or 122 swims per year for children and
1038 21 or 72 swims per year for a general population) are shown.

1039 Fig3

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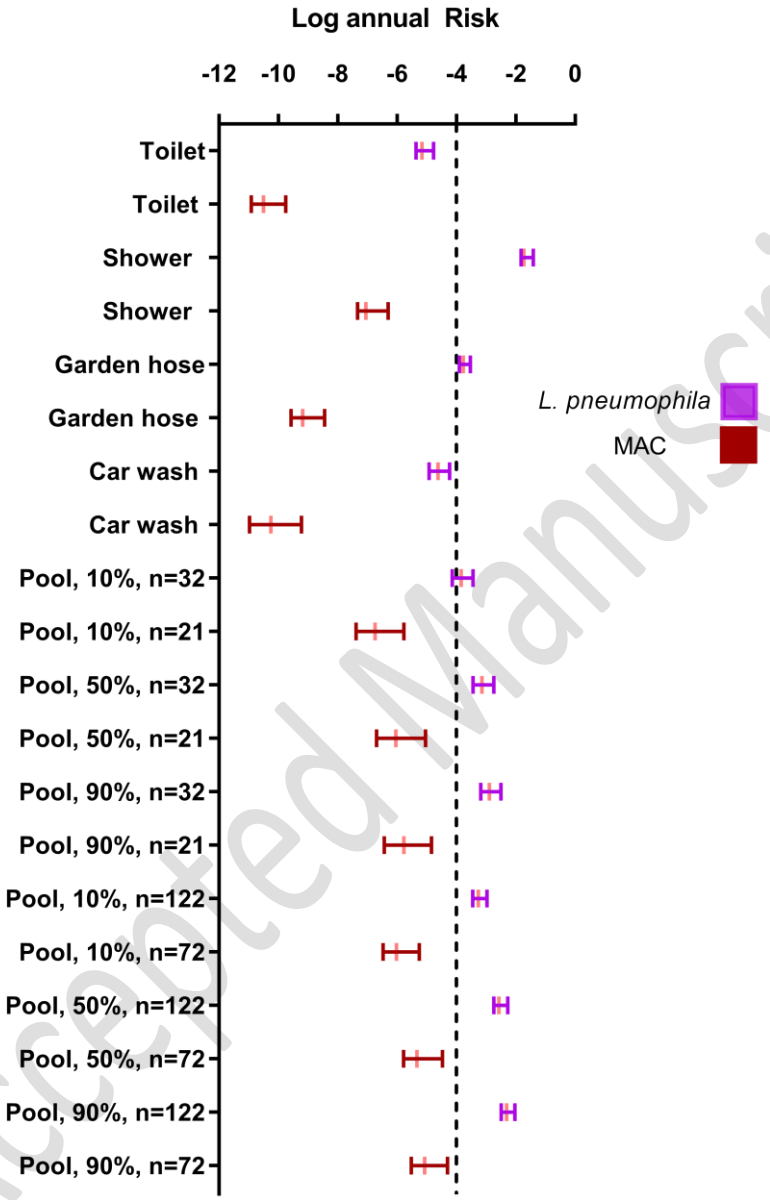

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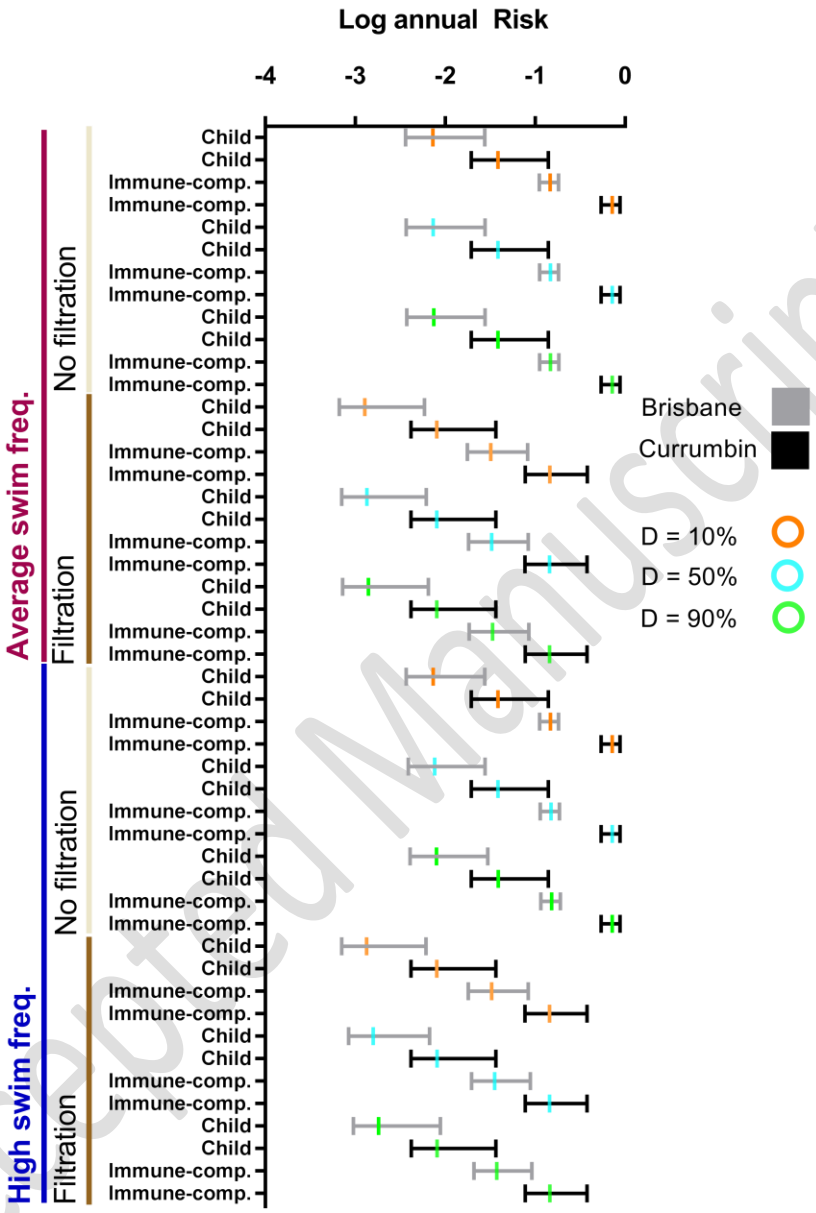
1043 **Fig. 3**. Annual infection risks for inhalation of *Mycobacterium avium* complex or L. *pneumophila* for
1044 various exposure scenarios. Median and 95% confidence intervals and scenario analysis for various pool
1045 dilution levels (D = 10%, 50%, or 90% RHRW: sterile water) and swimming assumptions (21 or 72 swims
1046 per for a general population) are shown.

1047

1048 Fig4



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1050

1051 **Fig. 4**. Total annual risks from all activities for cervical lymphadenitis in children or disseminated infection
1052 in immune-compromised populations via ingestion. Median and 95% confidence intervals shown.
1053 Scenario analysis for various pool dilution levels (D = 10%, 50%, or 90% RHRW: sterile water), with or
1054 without drinking water under-sink point of use filtration (filtration/no filtration), and swimming frequency
1055 assumptions (32 or 122 swims per year) are shown.

1056

1057 Fig5

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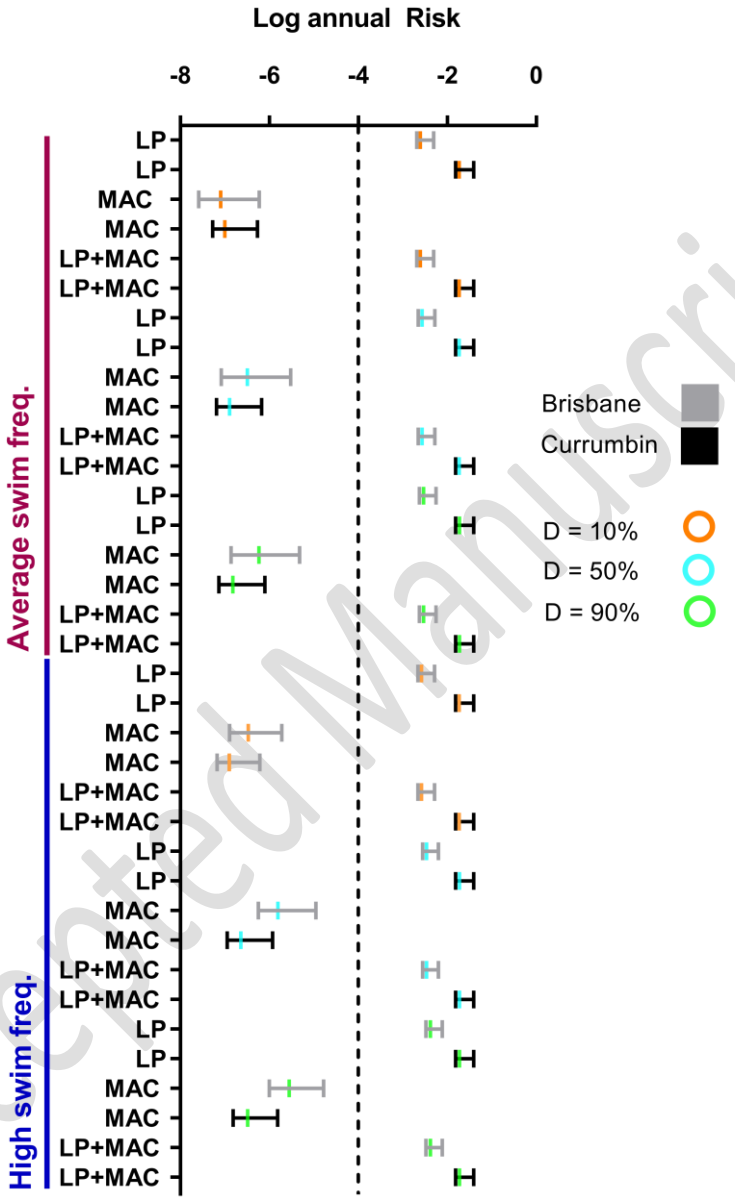

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1061 **Fig. 5**. Total annual pulmonary infection risks from all activities via inhalation of *Mycobacterium avium*
1062 complex (MAC), *L. pneumophila* (LP), or both organisms (LP+MAC). Median and 95% confidence
1063 intervals are shown. Scenario analysis for various pool dilution levels (D = 10%, 50%, or 90% RHRW:
1064 sterile water) and swimming assumptions (32 or 122 swims per year) are shown.

1065