MODELING RISK FOR INTRANASAL, INHALATION, AND CORNEAL EXPOSURES TO OPPORTUNISTIC PATHOGENS OF CONCERN IN DRINKING WATER

By

Kara Dean

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

Biosystems Engineering- Master of Science

2019

ABSTRACT

MODELING RISK FOR INTRANASAL, INHALATION, AND CORNEAL EXPOSURES TO OPPORTUNISTIC PATHOGENS OF CONCERN IN DRINKING WATER

By

Kara Dean

This study developed dose response models for determining the probability of eye, respiratory or central nervous system infections from previously conducted studies using *Naegleria fowleri*, *Acanthamoeba* spp. and *Pseudomonas aeruginosa*. These opportunistic pathogens have been identified in drinking water and premise plumbing systems, and a lack of dose response models for the appropriate exposure routes of concern has prevented researchers from quantifying the risk they pose to human health. Using the newly developed dose response model for *P. aeruginosa*, a reverse quantitative microbial risk assessment (QMRA) was completed to determine the threshold concentrations of *P. aeruginosa* associated with an annual risk of 10^{-4} for corneal and inhalation exposures. The results indicated that an average concentration of 1 CFU/L in the bulk water could result in an annual risk greater than the guideline set by the Environmental Protection Agency. The threshold concentration responsible for a 10^{-4} risk of pneumonia from *P. aeruginosa* was 11 orders of magnitude greater than the threshold concentration for bacterial keratitis. Modeling all possible exposure routes of concern for opportunistic pathogens in drinking water is critical, as the exposure route dramatically affects the concentrations of concern. This reverse QMRA and future risk assessments that utilize the dose response models developed in this study can be used to inform decisions on drinking water treatment, monitoring protocols, and future plumbing design.

ACKNOWLEDGMENTS

This thesis would not have been possible had I not been provided the opportunity to work with my advisor, Dr. Jade Mitchell. I would like to thank her for her guidance and support throughout my undergraduate and graduate years at Michigan State University. Her mentorship has inspired me to continue to pursue research that I am passionate about and I feel very lucky to be able to continue my education under her expertise.

I would also like to acknowledge my esteemed committee members: Dr. Joan Rose and Dr. Pouyan Nejadhashemi. Their input and assistance has made me a better researcher and has played an integral role in my professional development.

Thank you to my fellow graduate students and lab mates within the BE Department for their support, knowledge, and friendship.

Finally, I would like to thank my family for their tireless love and support. They have never failed to believe in me, making it much easier for me to believe in myself.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1: INTRODUCTION

It is a national priority to protect drinking water sources and ensure that safe drinking water is available to the public. In the United States, policies such as the Clean Water Act and Safe Drinking Water Act implemented by the Environmental Protection Agency (EPA) set quality standards, regulations, and guidelines to accomplish these goals. The National Primary Drinking Water Regulations are legally enforceable standards and treatment techniques for public water systems (Environmental Protection Agency, 2003). There are currently standards and techniques for 80 different disinfectants, disinfectant byproducts, inorganic chemicals, organic chemicals, and radionuclides. The number of microorganism or microorganism-related regulations is much smaller, with performance guidelines or treatment techniques existing only for *Cryptosporidium* spp., *Giardia lamblia*, *Legionella* spp., Heterotrophic Plate Counts (HPC), Total Coliforms, fecal coliforms and *E. coli*, enteric viruses, and turbidity (Environmental Protection Agency, 2003). Most of the microorganism-related regulations consist of required treatment techniques to reduce the level of the contaminant. The Safe Drinking Water Act also empowers the EPA to assess natural and man-made contaminants that may need to be regulated in the future through a three-step process: (1) the EPA evaluates contaminants that potentially threaten human health and prioritizes those to regulate; (2) a maximum contaminant level goal is determined; and, (3) a feasible standard for maximum contaminant levels is specified (Gerba, Nwachuku, & Riley, 2003; National Research Council, 1999).

Drinking water treatment continues to advance but waterborne-disease outbreaks associated with drinking water still occur from both groundwater and surface water supplied systems. From 2013-2014, 42 drinking water-associated outbreaks were reported in the U.S. that resulted in 1,006 cases of illness, 124 hospitalizations, and 13 deaths. *Legionella* was the

etiologic agent responsible for 24 of the outbreaks, 130 of the cases of illness and all of the deaths. *Cryptosporidium* or *Giardia* were responsible for eight outbreaks and 289 cases of illness and chemicals/toxins were associated with four outbreaks and 499 cases of illness (Benedict et al., 2017). Although water quality is monitored at the drinking water treatment facilities, there are opportunities for degradation and contamination once the water is distributed. The biological stability of the treated drinking water is threatened by the presence of pathogens and bacteria, either pre-existing or introduced, and the presence of nutrients and growth inhibitors throughout the system (Prest, Hammes, van Loosdrecht, & Vrouwenvelder, 2016).

Premise plumbing refers to the distribution of water beyond the property line and includes households, hospitals, and commercials buildings (Falkinham III, Hilborn, Arduino, Pruden, & Edwards, 2015). Premise plumbing systems facilitate the direct use of the water supply, and as such serve as the location where exposure to potentially contaminated water can occur. The way water quality changes once it is distributed to the premise is not thoroughly understood and there has been recent evidence that the number of waterborne opportunistic pathogens in premise plumbing are increasing (Joseph O. Falkinham, 2015). Pathogens such as *Legionella pneumophila*, *Mycobacterium avium*, *Pseudomonas aeruginosa*, *Naegleria fowleri* and *Acanthamoeba* spp. have been found in drinking water and drinking water associated biofilms (van der Wielen & van der Kooij, 2013; Wingender & Flemming, 2011b). These pathogens are microorganisms that naturally inhabit drinking water systems and can cause a variety of infections. Waterborne opportunistic pathogens, such as *Legionella*, have been identified as one of the leading causes of drinking water-associated waterborne disease outbreaks and understanding the degradation of water quality in pipe systems that lead to their occurrence and proliferation has been identified as a research priority (Benedict, 2017; Garner 2019).

In addition to the plumbing conditions influencing occurrence and growth, it is necessary to understand how exposures to these pathogens may occur through the use of premise plumbing and to quantify the risk they pose to human health. This can be achieved with quantitative microbial risk assessment (QMRA), a framework that is used to characterize the risk of waterborne pathogens (C. Haas, Rose, & Gerba, 2014). QMRAs consist of five main steps: hazard identification, dose response modeling, exposure assessment, risk characterization, and risk management. The QMRA framework integrates the knowledge of the pathogens of concern, the probability of adverse health outcomes associated with exposure doses, and the possible exposure routes and scenarios to characterize risk. Information provided by completed QMRAs can be used to inform risk management decisions for the design, treatment, and maintenance of water distribution systems.

RESEARCH GAPS

Previous risk assessments have been conducted of *L. pneumophila* and *M. avium* for premise plumbing exposures (Hamilton et al., 2018; Hamilton, Ahmed, Toze, & Haas, 2017; Schoen & Ashbolt, 2011). This has been facilitated by the existence of dose response models for the exposure routes of concern (Hamilton, Weir, et al., 2017; Schoen & Ashbolt, 2011). The risk posed by other opportunistic pathogens of note including *N. fowleri*, *Acanthamoeba* spp. and *P. aeruginosa* have not been evaluated partly because of a lack of published dose response models. Understanding the relationship between exposure dose and likelihood of occurrence of infection is a necessary step in conducting a QMRA. Although there are currently dose response models for the corneal route of exposure to *P. aeruginosa*, there is no model representative of an inhalation exposure (Tamrakar, 2013). Only an intravenous exposure has been modeled for *N. fowleri*, which does not represent the likely exposure route of concern (Y. Huang, 2013).

Dose response models for free-living amebae will help quantify the risk they pose individually, and facilitate future research into their relationships with other bacterial opportunistic pathogens. Although *L. pneumophila, M. avium*, and *P. aeruginosa* are three of the most commonly tracked opportunistic pathogens in premise plumbing, only *L. pneumophila* and *M. avium* have been assessed for possible in-home exposure scenarios (Falkinham III et al., 2015; Hamilton, Ahmed, et al., 2017; Schoen & Ashbolt, 2011). With the production of an inhalation dose response model for *P. aeruginosa*, QMRAs need to be conducted to understand the different exposure routes of concern and which exposure scenarios warrant the most risk management.

RESEARCH OBJECTIVES

In order to facilitate the production of future risk assessments, this study aims to develop dose response models for *N. fowleri*, *Acanthamoeba* spp., and *P. aeruginosa*. To further understand the importance of each exposure route for *P. aeruginosa*, a reverse QMRA will be conducted to determine the concentrations in the water responsible for an annual risk level of 10^{-4} for both corneal and inhalation exposure scenarios.

The specific objectives of this study are to: i) develop an intranasal dose response model for *N. fowleri*; ii) develop corneal and intranasal dose response models for *Acanthamoeba* spp.; iii) develop an inhalation dose response model for *P. aeruginosa*; and iv) complete a reverse QMRA of *P. aeruginosa* to compare risk posed by different exposure routes. The objectives were addressed in the form of four separate manuscripts entitled "Development of a Dose Response Model for *Naegleria fowleri*", "Dose Response Models for *Acanthamoeba* spp.", "A Dose Response Model for the Inhalation Route of Exposure to *P. aeruginosa*", and "Reverse QMRA for *P. aeruginosa* in Premise Plumbing to Inform Risk" that are Chapters 3, 4, 5, and 6,

respectively, in this thesis (Dean & Mitchell, 2019; Dean, Tamrakar, Huang, Rose, & Mitchell, 2019; Dean, Weir, & Mitchell, 2019). The results of this work will facilitate future risk assessments for all three pathogens and provide threshold concentrations of *P. aeruginosa* in the bulk water to be used to develop risk management strategies for drinking water.

CHAPTER 2: LITERATURE REVIEW

A literature review was conducted to identify the opportunistic pathogens of concern for human health, the conditions that lead to their survival in premise plumbing systems, and previous work done to assess the risk posed to human health through exposure at the tap.

OPPORTUNISTIC PATHOGENS OF CONCERN

Of the 42 waterborne disease outbreaks reported in the US between 2013 and 2015, *Legionella* spp. was the pathogen responsible 57% of the time (Benedict et al., 2017). *Legionella* spp. are gram-negative bacteria ubiquitous in water sources and have been identified in locations from source to tap throughout water distribution systems (Falkinham III et al., 2015; Lau & Ashbolt, 2009). The pathogen primarily affects the immunocompromised and causes Pontiac fever and Legionnaires' disease (Falkinham III et al., 2015). Pontiac fever is a mild, flu-like illness and Legionnaire's disease is a severe form of pneumonia. *Legionella pneumophila* serogroup 1 is primarily responsible for the majority of outbreaks (Lau & Ashbolt, 2009; Percival & Walker, 1999). Exposure to the pathogen occurs through the inhalation of contaminated (or bacterial laden) aerosols. Typical sources of aerosols include cooling towers, fountains, showers, and faucets (Lau & Ashbolt, 2009).

Mycobacterium avium complex (MAC) refers to a group of opportunistic pathogens that are the most common cause of clinically significant non-tuberculosis mycobacterium infections (Whiley, Keegan, Giglio, & Bentham, 2012). The prevalence of mycobacterial diseases, primarily caused by *M. avium*, is roughly 10 to 15 cases per 100,000 individuals in the U.S (Billinger et al., 2009; Falkinham III, 2013). The most common manifestation of non-tuberculosis mycobacterium infections is pulmonary disease. For the immunocompromised, MAC is known to cause pulmonary infections, gastrointestinal tract infections and skin

infections depending on the pre-existing conditions (Donohue et al., 2015; Whiley et al., 2012). MAC have been identified in the potable water of multiple countries including the US and it has been demonstrated that the bacteria are capable of growing and persisting within the distribution system (J.O. Falkinham, Norton, & Mark, 2001; Whiley et al., 2012). In terms of exposure to potable water, the exposure routes of concern are the inhalation of contaminated aerosols from showers, humidifiers or hot tubs, or through the ingestion of contaminated water by patients with severe immunodeficiency (Falkinham Joseph, 2013; Hamilton, Weir, et al., 2017).

Pseudomonas aeruginosa is another opportunistic pathogen known to proliferate in drinking water distribution systems (Trautmann, Lepper, & Haller, 2005). *P. aeruginosa* is a gram-negative bacterium responsible for a range of infections. It has been known to cause community- and hospital-acquired pneumonia, and chronic lung infections in patients with cystic fibrosis (Driscoll, Brody, & Kollef, 2007b; Kerr & Snelling, 2009). The morbidity and mortality rates of *P. aeruginosa* infections are elevated for the immunocompromised (Streeter & Katouli, 2016). In addition, the bacterium is also known to be one of the main etiologic agents of bacterial keratitis, a type of eye infection estimated to have an annual incidence of 20.9 and 4.1 cases per 10,000 persons using extended wear and daily wear contact lenses, respectively (Driscoll et al., 2007b; Kerr & Snelling, 2009; Poggio et al., 1989). These infections and routes of exposure make the pathogen's presence in drinking water particularly concerning, as exposure could occur through application of water to the eye or from the inhalation of aerosols.

In addition to the bacterial opportunistic pathogens mentioned, the free-living amebae (FLA) *Naegleria fowleri* and *Acanthamoeba* spp. are also considered opportunistic pathogens of concern. These FLA are concerns in drinking water not only because of their ability to cause harmful infections themselves, but also because they are known to harbor and protect other

opportunistic pathogens (J. M. Thomas & Ashbolt, 2010). *N. fowleri* is a FLA known to inhabit warm, freshwater sources and is responsible for the highly fatal infection, Primary Amebic Meningoencephalitis (PAM) (S. Kilvington & White, 1985; Ma et al., 1990). To initiate infection, the amebae has to be forcefully inhaled through the nose to facilitate access to the brain. There have been over a hundred cases in the United States, usually associated with swimming in warm freshwater sources, but also from exposure to treated drinking water (CDC 2008). For example, in Louisiana there was a fatal case of PAM from the use of contaminated tap water in a neti-pot (J. R. Cope et al., 2015). *Acanthamoeba* spp. is another type of FLA that has been detected in drinking water distribution systems. *Acanthamoeba* spp. are responsible for *Acanthamoeba* keratitis, an eye infection, and the rare CNS infection, Granulomatous Amebic Encephalitis (GAE) (Marciano-Cabral & Cabral, 2003). *Acanthamoeba* spp. has also been shown to associate and increase the survival ability of *Legionella* in drinking water (Bichai, Payment, & Barbeau, 2008; Lau & Ashbolt, 2009).

CONDITIONS PROMOTING MICROBIAL GROWTH IN PREMISE PLUMBING SYSTEMS

Temperature cycling, storage and stagnation, biofilm presence, and decaying disinfectant residuals are all conditions that are conducive for the proliferation of pathogens within the premise plumbing system (J. Falkinham, Pruden, & Edwards, 2015). Within the premise plumbing system, temperature setting has been a main factor driving changes in opportunistic pathogen abundance and microbial community composition (Bédard et al., 2015; Dai, Rhoads, Edwards, & Pruden, 2018). Water heaters provide an element of treatment by heating the water, however it is impossible to maintain that water heater set point throughout the system, and warm water temperatures (32-41°C) have been shown to stimulate microbial growth (Bédard et al., 2015; Dai et al., 2018; Rhoads, Ji, Pruden, & Edwards, 2015). The World Health Organization

(WHO) recommends setting water heaters to 60°C to limit pathogen growth (World Health Organization, 2007). This recommendation is not often followed, however, because 60°C poses a scalding risk. The EPA thus suggests a setting of 49°C as a safer, more energy efficient choice (Brazeau & Edwards, 2013). *Legionella* spp. can not survive temperatures above 50°C but when *L. pneumophila* grows within free-living amebae, it has additional protection from temperatures and treatment (Lau & Ashbolt, 2009; Percival & Walker, 1999). *P. aeruginosa* can grow between 10 and 42°C, with an optimum growth temperature of 37°C (Bédard, Prévost, & Déziel, 2016). These temperatures are below the EPA recommended water heater setting, however *P. aeruginosa* has also been known to interact with free-living amebae which can allow it to survive temperatures above 55°C (Bédard et al., 2016; S. Cervero-Aragó, Rodríguez-Martínez, Canals, Salvadó, & Araujo, 2013). Although temperature increases to 60°C and above have shown to decrease *P. aeruginosa* contamination, thermal disinfection has not been proven to be effective at eradication once the pathogen has already colonized the system (Bédard et al., 2016). Homes with high hot water temperatures have shown to have a lower number of *M. avium* (J. Falkinham et al., 2015). A study of free-living amebae under temperature treatment identified a difference in persistence between trophozoites and cysts. Treatment at 50°C reduced trophozoite viability by 2-3 $\log_{10}$, but cysts by less than 1 $\log_{10}$. Treatments at 60°C and 70°C were much more effective and indicate that the common water heater settings may not be adequate to reduce FLA concentration (S. Cervero-Aragó et al., 2013).

Biofilms are present on all the surfaces involved in water treatment, distribution and storage, and these surfaces are usually more highly colonized than the bulk water (Flemming, Wingender, & Szewzyk, 2011). Biofilm presence in premise plumbing systems provides nutrients and protection that allow for greater pathogen growth (WHO, 2007). The presence,

persistence, and multiplication of *Legionella* spp. in biofilms has been observed on a variety of piping materials and over a range of temperatures (Flemming et al., 2011). Previous studies have indicated that *P. aeruginosa* amplifies within the premise plumbing or the tap rather than within the main water distribution system, and presence of *P. aeruginosa* is strongly correlated to biofilm colonization of point-of-use devices such as faucets, drains, and showerheads (Bédard et al., 2016). Unlike *Legionella* spp., MAC species are not dependent on free-living amebae within the biofilm for replication (Flemming et al., 2011). *M. avium*, *M. intracellulare*, and *M. abscessus* have all been shown to readily adhere and form biofilms on stainless steel, glass, zinc-galvanized steel, copper and polyvinyl chloride (Mullis & Falkinham, 2013). Considering amebae feed on bacteria, it is likely that a biofilm is the preferred habitat for free-living amebae as well. A study of dental unit water identified concentrations of amebae 300 times greater from the dental unit water than the tap water. The ratio of area to volume of the water lines was 6:1, providing a large surface for biofilm colonization (Barbeau & Buhler, 2001). The biofilm also aids in the pathogens' persistence. *N. fowleri* has been shown to be able to survive chlorine concentrations 30 times (20 mg/L for 3 hours) than the recommended amount when established in attached biofilms (Miller et al., 2015).

The concentrations of chlorine and other disinfectant residual types is an element of concern within premise plumbing systems. Although chemical treatments such as monochloramine, chlorine and chlorine dioxide have been shown to be effective at controlling the growth of pathogens like *Legionella* and *Acanthamoeba*, their effectiveness can be strain dependent and it is a concern that the necessary residual levels are not always maintained (Dupuy et al., 2011; Kim, Anderson, Mueller, Gaines, & Kendall, 2002). This decay may be highly dependent on the piping materials used, as greater decay has been observed in copper

pipes than galvanized iron or polyvinyl chloride (PVC) (Zheng, He, & He, 2015). Piping material is another controversial factor affecting pathogen growth in plumbing systems as studies have had contradicting results. Although plastic piping such as PVC may maintain free chlorine residuals better than other metal piping types, some studies have shown it may be more prone to biofilm growth. In one study polymer piping had greater biofilm formation intensity than stainless steel and allowed for more *Legionella* spp. growth due to a greater leaching of nutrients and pre-existing hollows from the manufacturer (Rogers J., Dowsett A.B., Dennis P.J., Lee J.V., & Keevil C.W., 1994). Imperfections in the material as a location for biofilm formation and persistence was also demonstrated in a study of polyethylene water storage tanks (Van Der Merwe, Duvenage, & Korsten, 2013). *M. avium* amounts dramatically increased in water and biofilms on PEX, suggesting the material is more supportive of biofilm structures that allows pathogens to persist and grow (Bukh & Roslev, 2014; J.O. Falkinham et al., 2001; Lu et al., 2014). Ultimately, although there are several factors affecting the growth and proliferation of opportunistic pathogens and free-living amebae, the main influencers within premise plumbing systems include temperature changes, stagnation, biofilm development, and decaying disinfectant residuals.

QUANTITATIVE MICROBIAL RISK ASSESSMENT AND OPPORTUNISTIC PATHOGENS

Premise plumbing systems are more prone to stagnant flow conditions and as such, biofilm development and chlorine residuals decay. As water use trends continue to decline, stagnation within the systems will grow. Before new treatment protocols or management practices can be designed, the concentrations of opportunistic pathogens in the water that pose a risk to human health need to be determined. Quantitative microbial risk assessment (QMRA) is a

framework used to characterize the risk of waterborne pathogens (C. Haas et al., 2014). The results of a QMRA can be used to inform risk management decisions for the design, treatment, and maintenance of water distribution systems. QMRAs consist of five main steps: hazard identification, dose response modeling, exposure assessment, risk characterization, and risk management.

Previous QMRAs have been conducted for *L. pneumophila* and *M. avium* in terms of premise plumbing system exposure (Hamilton et al., 2018; Hamilton, Ahmed, et al., 2017; Schoen & Ashbolt, 2011). An in-premise model determined bacterial densities of *L. pneumophila* in the air, water, and biofilm that could result in an infection during a 15-minute showering event. The range of concentrations in the water was calculated to be from $3.5x10^6$ to $3.5x10^8$ CFU/L (Schoen & Ashbolt, 2011). Health risks from exposure to *Legionella* were also evaluated for toilet flushing and it was determined that the median annual infection risks exceeded 10^{-4}, however, this risk was highly dependent on the assumptions made about how *Legionella* was partitioned in aerosol (Hamilton et al., 2018).

Another QMRA investigated the risks of using roof-harvested rainwater and assessed exposure scenarios such as car washing, toilet flushing and garden hose use for *L. pneumophila*, and drinking, accidental ingestion and inhalation for MAC. Risk was highest for the drinking exposure route and the risk of infection from inhalation was 6 orders of magnitude higher for *L. pneumophila* than for MAC (Hamilton, Ahmed, et al., 2017). Median annual risks for *L. pneumophila* exceeded 10^{-4} for the showering and garden hose exposures, and for all MAC exposure scenarios, the 95% confidence intervals were below the 10^{-4} benchmark. This study did not use a partitioning coefficient for the inhalation exposures to *L. pneumophila* and instead quantified the different volumes of specific aerosol sizes, further emphasizing the importance of

further elucidating the way the pathogen aerosolizes to accurately capture risk (Hamilton, Ahmed, et al., 2017).

CHAPTER 3: DOSE RESPONSE MODEL FOR *NAEGLERIA FOWLERI*

INTRODUCTION

Free-living amebae (FLA) are present in freshwater sources. *Naegleria fowleri* is a thermophilic FLA commonly found in warm freshwater bodies and can survive temperatures of up to 40-45 °C (S. Kilvington & White, 1985; Ma et al., 1990). *N. fowleri* is responsible for Primary Amebic Meningoencephalitis (PAM), a highly fatal infection. The infection is mostly acquired through forceful entry of water with the amebae into the nasal canal such that the amebae is able to migrate to the brain. This form of forceful inhalation into the nose could occur while swimming or diving in a body of water or bath, or perhaps with the use of a neti-pot (Bright & Gerba, 2017). Onset is rapid, with symptoms beginning with headache, fever, and nausea and quickly escalating to coma and seizures (Ma et al., 1990). PAM has a fatality rate of about 98%, affecting mostly children or young adults that have spent time swimming (Bartrand, Causey, & Clancy, 2014; CDC, 2011). In a study conducted by the CDC, 121 cases of PAM were reviewed from 1937-2007 and the majority of exposures occurred in warm, freshwater sources in the southern states of the U.S. (CDC, 2008).

Although most incidences of PAM are seen in cases of swimming in warm waters, infections associated with potable water have also been reported (Blair, Sarkar, Bright, Marciano-Cabral, & Gerba, 2008; Ma et al., 1990). Some non-swimming cases reported were associated with contaminated premise plumbing systems or improper neti-pot usage (Bartrand et al., 2014). *N. fowleri* was present on both the third and fourth contaminant candidate lists (CCL) published by the Environmental Protection Agency because despite generally low outbreaks and occurrences, the health effects from infection are severe (Hoffman, Marshall, Gibson, & Rochelle, 2009; U.S. EPA, 2009; U.S. EPA, 2016). Concern over the colonization of Arizona

wells by *N. fowleri* prompted a study where PCR detected *N. fowleri* DNA in 11 of the 143 tested wells (Blair et al., 2008).

In an effort to conserve water and pursue Green building designs, low flow conditions are becoming more common in water distribution systems. This is of particular concern with *N. fowleri* because it gains resistance against different treatment strategies when associated with biofilms. In a study conducted by Miller et al. (2015), *N. fowleri* established in attached biofilms were able to survive chlorine concentrations 30 times greater than the recommended amount (20 mg/L for 3 h and 10 mg/L for 48 h). The risk posed by *N. fowleri* to drinking water distribution systems may increase as the result of warmer temperatures from changing climate conditions and lower flows in plumbing systems. To better understand the risk posed by *N. fowleri* in these systems, Quantitative Microbial Risk Assessments (QMRA) are needed. QMRA is a widely used framework for risk characterization of waterborne pathogens in order to inform decisions about treatment, alternative design and selection (C. Haas et al., 2014).

A dose-response model to establish the mathematical relationship between exposure dose and risk for *N. fowleri* is needed for the forceful inhalation exposure route. This study aims to develop dose-response models from previously conducted studies on *N. fowleri* in the laboratory setting (D.T. John & Nussbaum, 1983; David T John & Hoppe, 2017). With these dose response models, QMRAs can be performed to further inform the future design and treatment of drinking water distribution systems.

MATERIALS AND METHODS

Data

John and Hoppe determined patterns of susceptibility for small wild mammals exposed to *N. fowleri* (David T John & Hoppe, 2017). *N. fowleri* was instilled intranasally into a single naris of oppossums, raccoons, squirrels, muskrats, rabbits, mice and rats using an Eppendorf pipet, and it was determined that mice were the most susceptible to infection. Male and female mice (10 in each group) were intranasally inoculated with the LEE strain of *N. fowleri* at doses ranging from 1,000 to 1,000,000 amebae per mouse. Amebae were grown in Nelson's medium and incubated at 37°C. Exponential growth phase amebae were harvested with centrifugation and inoculum was adjusted to desired cell concentrations. Experiment 1 in Table 3.1 shows dose response results from this study.

John and Nussbaum (1983) studied the infection acquired by mice through swimming in amebae-contaminated water (D.T. John & Nussbaum, 1983). Groups of 10 CD1 mice were placed in a one liter volume of distilled water containing different doses of amebae of the LEE strain of *N. fowleri* per ml of distilled water. Mice can normally float and keep their heads above water. To simulate an actual swimming exposure, groups of mice were put in the same container to create a crowded environment to spur swimming activity. After a specific time of swimming exposure (2.5, 5, 10 and 20 minutes), the mice were removed from the water and dried. The cumulative percentage of dead animals was recorded up to 28 days after exposure (D.T. John & Nussbaum, 1983). The concentrations in the water for the 5, 10, and 20 minute studies were analyzed and are shown in Table 3.1 as Experiment 2, 3, and 4, respectively.

Table 3.1: Dose Response Experiment Data for *N. fowleri*

Experiment	Exposure	Endpoint	Dose (no. of organisms)*	Responses			Resource
				Positive	Negative	Total	
1	Intranasal	Death	1,000	7	3	10	John and Hoppe (1990)
			10,000	8	2	10	
			100,000	10	0	10	
			1,000,000	10	0	10	
2	Swimming for 5 min.	Death	100	0	10	10	John and Nussbaum (1983)
			1,000	0	10	10	
			10,000	1	9	10	
			100,000	4	6	10	
			1,000,000	7	3	10	
3	Swimming for 10 min.	Death	100	0	10	10	John and Nussbaum (1983)
			1,000	1	9	10	
			10,000	4	6	10	
			100,000	6	4	10	
4	Swimming for 20 min.	Death	100	0	10	10	John and Nussbaum (1983)
			1,000	1	9	10	
			10,000	4	6	10	
			100,000	7	3	10	

*For Experiments 2, 3, and 4, the model is first fit to the concentration with units of amebae/mL

Analysis Methods

The data were evaluated against specific quality criteria before modeling. This criterion consisted of ensuring that (1) three or more graded doses were administered in the experiments; (2) at least three animals were tested in each dosing group; and (3) the data had a statistically significant trend by the Cochran-Armitage test (C. N. Haas, Rose, & Gerba, 2014; Neuhäuser & Hothorn, 1999). The studies evaluated included an adequate description of the dose

administered, strain of the pathogen, host species, number of positive responses and number of negative responses. Previously developed computer code in the statistical programming language, "R" (www.r-project.org) (Weir, Mitchell, Flynn, & Pope, 2017) was used to fit the dose-response models using the method of maximum likelihood estimation (MLE) as described in Haas et al. (2014). Both the exponential dose-response model (Equation 1) and the approximate form of the beta-Poisson dose-response model (Equation 2) were fit to the data (C. Haas et al., 2014).

The exponential dose-response model is given by Equation (1) where *P(d)* is the probability of response at dose, *d* , and the single parameter, *k*, is optimized during fitting and represents the probability that a single organism can survive to initiate the observed response.

$$P(d) = 1 - e^{-kd} \tag{1}$$

The approximate beta-Poisson model is given by Equation (2) where N_{50} is the median infective dose and α is a shape parameter (C. Haas et al., 2014; Teunis & Havelaar, 2000). In this study, the N_{50} is actually an LD_{50}, the median lethal dose because the observed response in all data sets was death.

$$P(d) = 1 - [1 + \left(\frac{d}{N_{50}}\right) * \left(2^{1/\alpha} - 1\right)]^{-\alpha} \tag{2}$$

The "rule of thumb" from Xie et al. (2017) was used to validate the application of the approximate beta-Poisson. These researchers propose $Pr(0<r<1)|\hat{\alpha}, \hat{\beta}) > 0.99$ as a validity measure for the appropriate use of the approximate beta-Poisson with the constraint $\hat{\beta} > (22\hat{\alpha})^{0.50}$ for $0.02 < \hat{\alpha} < 2$ (Xie et al., 2017). This methodology was used to validate the use of the approximate beta-Poisson for all four data sets in this study.

To establish goodness of fit for the models, a comparison of the optimal value of the deviance to the critical χ^2 value at degrees of freedom equal to the number of doses minus the number of fitted parameters at an alpha value of 0.05 was conducted as previously described by Haas et al. (2014). In order to compare the fit of the two models for each data set, an assessment of the statistical significance of improvement of fit was made by comparing the reduction in minimized deviance with the critical χ^2 value at 1 degree of freedom between the two-parameter beta-Poisson model and the one-parameter exponential model. Confidence bands were estimated using a bootstrapping resampling technique.

When multiple data sets were available for the same pathogen, a statistical pooling analysis was performed to ascertain whether the data set had the same underlying distributions. A likelihood ratio test was used to determine if data could be pooled.

RESULTS

N. fowleri injected to CD1 mice through nasal cavity

The beta-Poisson was the best fit model for the CD1 mice exposed intranasally to *N. fowleri* in Experiment 1. The minimized deviance of the exponential model exceeded the χ^2 value at degree of freedom one while the beta-Poisson model was well within the critical χ^2 value. Moreover, differences in deviances provided statistical significance of improvement of the beta-Poisson over the exponential model. The statistics of the two model fits to the animal studies are summarized in Table 3.2 and the best-fit model is shown in Figure 3.1. Due to the lack of singular responses, confidence bands could not reliably be estimated for the model. Singular responses refer to the responses in the dosing study where all or none of the subjects showed a response. In Experiment 1, there were no doses where none of the subjects exhibited a response.

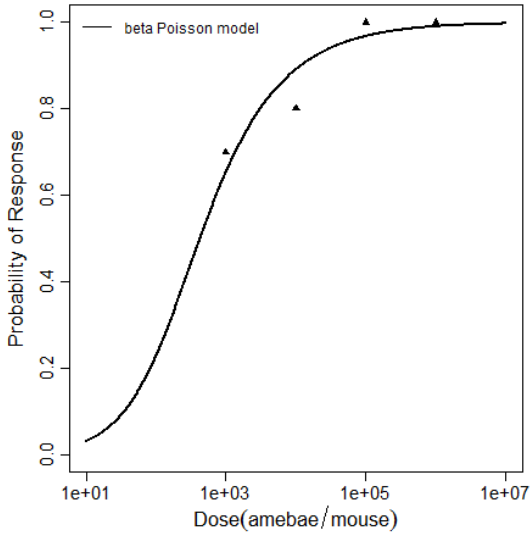


Figure 3.1 Plot of beta-Poisson model for CD1 mice exposed intranasally to *N. fowleri*

N. fowleri inhaled by swimming mice

The beta-Poisson model provided the best fit for the CD1 mice exposed to different concentrations of amebae in the water while swimming in Experiments 2, 3 and 4. The beta-Poisson models for each experiment are shown below.

Swimming for 5 minutes

The minimized deviance of the exponential model provided acceptable fit but difference in deviances (5.88) provided statistical significance of improvement of the beta-Poisson over the exponential model. The statistics of the two model fits to the animal study are summarized in Table 3.2 and the best-fit model with confidence bands is shown in Figure 3.2.

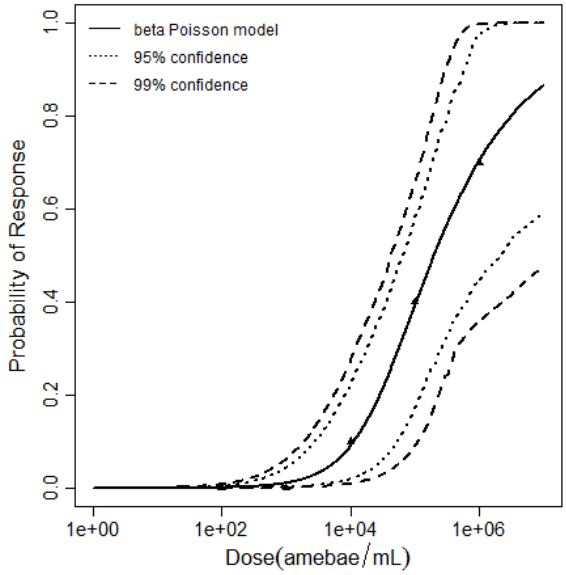


Figure 3.2 Plot of beta-Poisson model for CD1 mice exposed via swimming for 5 minutes with upper and lower 95% and 99% confidence

Swimming for 10 minutes

The beta-Poisson model was the best fit for the CD1 mice that swam for 10 minutes. The minimized deviance of the exponential model (7.88) exceeded the χ^2 value (7.81) at degree of freedom one and that of the beta-Poisson model was well within the critical value. Moreover, difference in deviances (7.55) provided statistical significance of improvement of the beta-Poisson over the exponential model. The statistics of the two model fits for Experiment 3 are summarized in Table 3.2 and the best-fit model with confidence bands is shown in Figure 3.3.

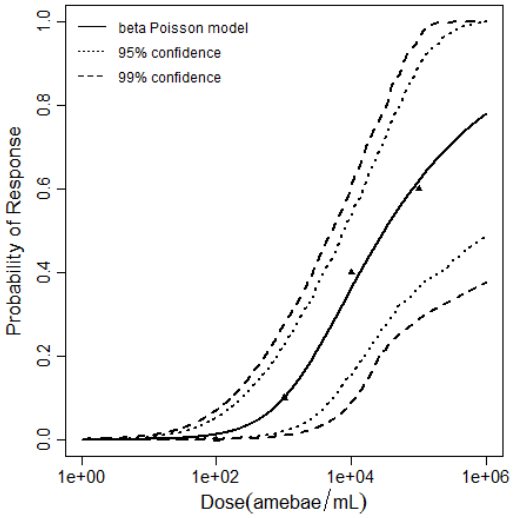


Figure 3.3 Plot of beta-Poisson model for CD1 mice exposed via swimming for 10 minutes with upper and lower 95% and 99% confidence

Swimming for 20 minutes

The minimized deviances of both the exponential as well as the beta-Poisson model provided acceptable fits. However, a difference in deviances (5.898) provided statistical significance of improvement of the beta-Poisson over the exponential model. The statistics of the two model fits are summarized in Table 3.2 and the best-fit model with confidence bands is shown in Figure 3.4.

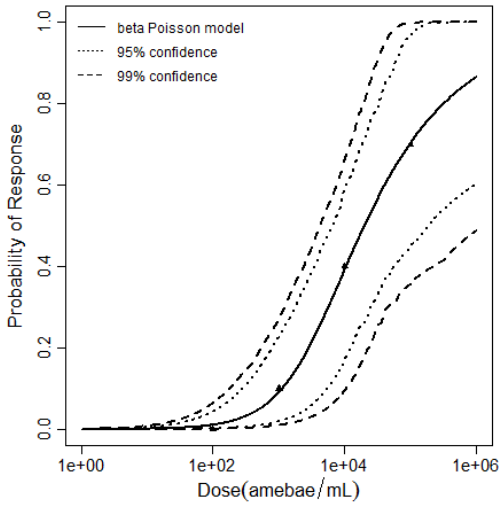


Figure 3.4 Plot of beta-Poisson model for CD1 mice exposed via swimming for 20 minutes with upper and lower 95% and 99% confidence

Table 3.2: Statistics for *N. fowleri* Dose Response Models

Experiment	Model	Deviance	Δ	DF	$\chi^2_{\alpha,n-k}$	$\chi^2_{\alpha,1}$	Best Fit	Parameters	LD_{50}
1	Exponential	11.28	9.64	3	7.81	3.84	beta-Poisson	α=0.536 N_{50}=422.05	422
	beta-Poisson	1.64		2	5.99				
2	Exponential	6.13	5.88	4	9.49	3.84	beta-Poisson	α =0.352 N_{50}=198,602	198,602
	beta-Poisson	0.25		3	7.81				
3	Exponential	7.89	7.55	3	7.81	3.84	beta-Poisson	α =0.241 N_{50}=30,447	30,447
	beta-Poisson	0.33		2	5.99				
4	Exponential	6.13	5.90	3	7.81	3.84	beta-Poisson	α =0.350 N_{50}=19,805	19,805
	beta-Poisson	0.23		2	5.99				

Pooling analysis

Data of swimming episodes of CD1 mice for three different time periods (5 min, 10 min and 20 min) in different concentrations of amebae per mL could be pooled. The value of difference in deviances between the sum of the individual best fits and pooled best fit was 6.584, which was less than the $\chi^2_{0.05,4}$ value (9.487). The summary and statistics of the pooling analysis

are shown in Table 3.3 and Figure 3.5. The confidence of the bootstrapped parameters is shown in Figure 3.6.

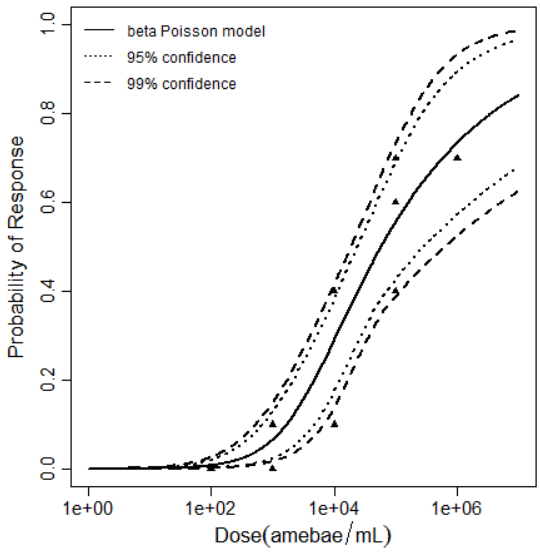


Figure 3.5 Plot of beta-Poisson model for pooled data of CD1 mice swimming for 5, 10 and 20 minutes with upper and lower 95% and 99% confidence

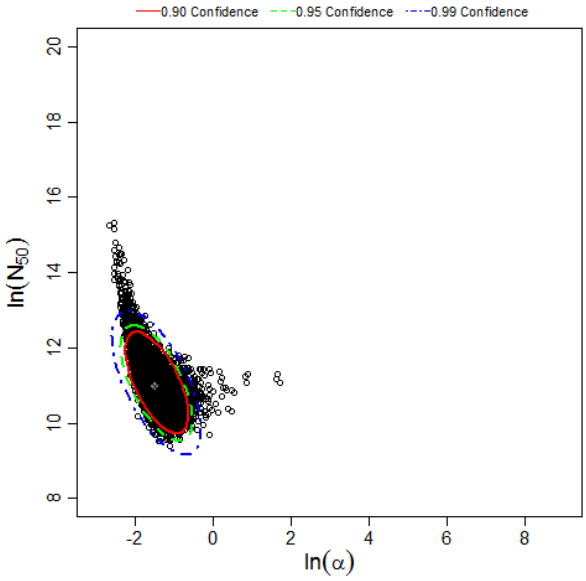


Figure 3.6 Bootstrapped distribution of beta-Poisson parameter estimates for pooled data of CD1 mice swimming for 5, 10 and 20 minutes; the center marker (X) represents the maximum likelihood estimate

Table 3.3: Pooling Statistics for *N. fowleri*

Data Set	Number of Doses	Best Fit Model	Minimized Deviance	DF	$\chi^2_{\alpha,n-k}$	$\chi^2_{\alpha,1}$	Parameters	LD_{50}
Pooling 2, 3, and 4 (Concentrations and Doses*)	13	beta-Poisson	7.40	11	19.68	0.77	α=0.226 N_{50}=57,938	57,983
							α*=0.226 N50*=13,257	13,257*

Final recommended model

For the recommended dose response model for exposure to *N. fowleri* in a swimming event, the pooled concentrations were transformed into exposure doses. Previous studies with mice determined an average breathing rate of 261 breaths/minute and a tidal volume of 0.16 mL (Karrasch, Eder, Bolle, Tsuda, & Schulz, 2009). The method for experimental drowning of rats administers water intratracheally at a rate of 1 mL/minute until cardiac arrest occurs. In previous experiments, cardiac arrest occurred in under 3 minutes (Locali, Almeida, & Oliveira-Júnior, 2006). The total lung capacity of rats is approximated to be 10 times greater than that of mice (Irvin & Bates, 2003). Applying this ratio to the rodent drowning procedure approximates the volume that could cause death in a mouse to be 0.30 mL. Since the mice in John and Nussbaum's study survived the swimming event, it can be assumed that they did not inhale the 0.30 mL of water necessary for cardiac arrest to occur. Thus, it was assumed that each mouse only inhaled water or water aerosols for a maximum of 2 breaths during their swimming event. With the two breaths, average tidal volume, and a retention rate in the nasal region of 71.5%, the inhaled dose of amebae was calculated and the beta-Poisson model was the best fit with a deviance of 7.40 (Raabe, Al-Bayati, Teague, & Rasolt, 1988). The statistics of the models are shown in Table 3.3 and the model with confidence bands and the bootstrapped parameter distributions are shown in Figures 3.7 and 3.8, respectively.

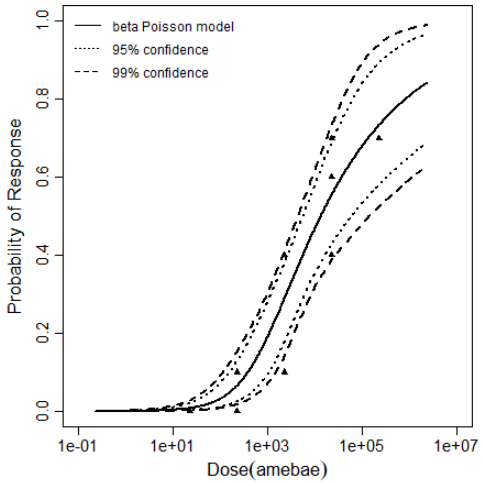


Figure 3.7: Recommended beta Poisson dose response model for CD1 mice exposed to amebae in a swimming event with upper and lower 95 and 99% confidence

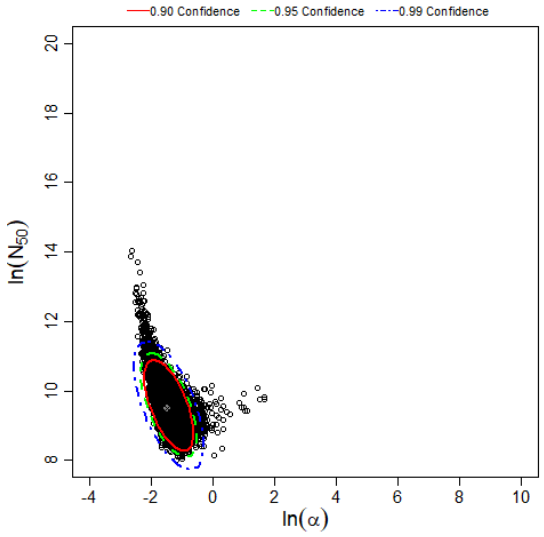


Figure 3.8 Bootstrapped distribution of beta-Poisson parameter estimates for pooled data of CD1 mice swimming for 5, 10 and 20 minutes; the center marker (X) represents the maximum likelihood estimate

DISCUSSION

The studies by both John and Hoppe (1990) and John and Nussbaum (1983) evaluated intranasal exposure to the LEE strain of *N. fowleri* in CD1 mice. John and Hoppe (1990) inoculated intranasally into a single naris of immobilized animals using Eppendorf pipettes, while John and Nussbaum (1983) placed the animals into one liter volumes of distilled water containing specified numbers of amebae to provide a tumultuous environment for swimming.

The best fit model for the CD1 mice exposed intranasally via pipette and CD1 mice swimming at different time periods is the beta-Poisson, indicative of a heterogeneous response.

The beta-Poisson curve for the direct intranasal inoculation was steeper than the curves for the previously conducted dose-response study on intravenous exposure to *N. fowleri* (Huang, 2013). This is indicative of a higher delivered dose reaching the receptive tissue, resulting in a higher probability of death. The LD_{50} for the intranasal inoculation was the lowest of the four experiments assessed in this study with a value of 422 ameba. This exposure route being more lethal is logical considering *N. fowleri* infection is associated with contaminated water being forced into the nasal cavity, an action that does not always naturally occur when just swimming. In Experiments 2-4, multiple mice were crowded into a single container to create a violent swimming environment where inhalation of water through the nose could occur. The individual model of different time periods shows the longer the swimming period, the lower the LD_{50} and the higher the probability of death for each dose. However, as the data sets of all the swimming periods could be pooled, this indicates that all the swimming episode cases can be described by a single model and would be considered mechanistically similar.

The pooled and final recommended model shown in Table 3.3 and Figure 3.7 had an LD_{50} of ameba. The previously completed dose-response model for *N. fowleri* was completed with an intravenous exposure. The best-fit model for the intravenous exposure was the exponential model with an LD_{50} of 2,030,000 (Huang, 2013). The model recommended in this study more accurately estimates the likelihood that *N. fowleri* ameba reaches the target receptor to initiate infection.

CONCLUSIONS

The dose-response models developed in this analysis are the first step in quantifying the risk *N. fowleri* poses to the population when present in drinking water distribution systems because they more closely match the expected exposure route. Although there are infrequent outbreaks and occurrences, the deadly effects of infection make *N. fowleri* a drinking water regulation focus (Hoffman et al., 2009). Although *N. fowleri* can be controlled with chemical and physical implementations in the premise plumbing system, it is the faltering or evading of these systems that causes concern. *N. fowleri* found in an area of a treated drinking water system with no detectable total chlorine residuals and temperatures greater than 30 °C was linked to a fatal infection in the United States (J. Cope et al., 2015). The recommended dose response model developed in this study can be used to perform QMRAs to help risk managers protect human health and properly manage drinking water distribution systems to ensure safe drinking water.

CHAPTER 4: DOSE RESPONSE MODELS FOR *ACANTHAMOEBA* SPP.

INTRODUCTION

Free-living amoeba (FLA) naturally exist in drinking water distribution systems. Some FLA are pathogenic, and some serve as hosts for other, more harmful pathogens. The interactions between FLA and amoeba-resisting microorganisms like opportunistic pathogens, can possibly occur throughout all stages of the water distribution system (Hoffmann & Michel, 2001; J. M. Thomas & Ashbolt, 2010). There have been recorded incidences of FLA interacting with opportunistic pathogens in premise plumbing such as *Legionella*, *Mycobacterium*, and *Pseudomonas* (J. M. Thomas & Ashbolt, 2010; V. Thomas, Blanc, Bille, & Greub, 2005). Opportunistic pathogens naturally inhabit the infrastructure and can pose a threat to certain populations, especially the immunocompromised. The target populations include the elderly and those with preexisting health conditions (J. Falkinham et al., 2015). When these pathogens become incorporated in FLA like *Acanthamoeba*, they become more capable of surviving barriers such as higher temperatures and disinfection. In addition to offering protection, the amebae have been shown to aid in multiplication, transportation and increasing virulence potential of the bacterial pathogens (Bichai et al., 2008).

FLA have been detected in drinking water distribution systems around the world and a comprehensive measurement of the risk they pose to human health is a source of uncertainty (J. M. Thomas & Ashbolt, 2010). *Acanthamoeba*, a type of FLA, has been shown to increase the chance of survival for associated *Legionella* under the pressures of treatment (Sílvia Cervero-Aragó, Rodríguez-Martínez, Puertas-Bennasar, & Araujo, 2015). Not only can FLA serve as hosts that provide additional protection to premise pathogens but some are threats to human health as individuals. *Acanthamoeba spp.* are commonly found in freshwater, recreational water,

tap water, and heating and cooling units. *Acanthamoeba spp*. can cause eye infections, skin lesions, or Granulomatous Amebic Encephalitis (GAE) (Marciano-Cabral & Cabral, 2003). Specifically, *Acanthamoeba* keratitis is most commonly associated with contact lens usage and is a sight-threatening corneal disease (Marciano-Cabral & Cabral, 2003). Unlike other *Acanthamoeba* infections, *Acanthamoeba* keratitis is not limited to immunocompromised hosts, and caused about 3000 infections in the year 2004 (F. L. Schuster & Visvesvara, 2004; J. M. Thomas & Ashbolt, 2010). The estimated annual incidence of *Acanthamoeba* keratitis in developed countries is roughly estimated to be between one and 33 per million contact lens users (Yoder et al., 2012). Exposure to these amebae can occur through household use of drinking water. A study done in Ohio found FLA in 79% of the 467 households studied. *Acanthamoeba* was specifically found in 51% of the homes' water samples (Stockman, Wright, Visvesvara, Fields, & Beach, 2011).

In order to develop adequate and effective water management plans, it is important to understand the human health risk associated with FLA like *Acanthamoeba*. A Quantitative Microbial Risk Assessment (QMRA) is a widely used framework for the risk characterization of waterborne pathogens in order to inform decisions about treatment and design alternatives for protection of public health (C. Haas et al., 2014). The use of QMRA necessitates a pathogen specific dose response model to describe the mathematical relationship between the probability of an infection as measured by replication, disease or an immunological response and a given exposure dosage of organisms through a specific exposure route. Addressing FLA and their impact on the virulence of other bacterial pathogens is a noted gap.

Before developing QMRA models to inform risk management strategies, it is important to first address the dose response models for *Acanthamoeba*. This study aims to develop dose

response models from previously conducted studies on the infection of animals with *Acanthamoeba* Ac 118, *Acanthamoeba castellanii,* and the *Acanthamoeba castellanii* HN-3 (Paul R. Badenoch, Johnson, Christy, & Coster, 1990; Cerva, 1967a, 1967b; Culbertson, Ensminger, & Overton, 1966). This work sets the stage for developing more accurate risk assessments for other premise plumbing pathogens and their interactions with FLA in future works.

MATERIALS AND METHODS

The previously conducted dose response studies evaluated corneal, intranasal, intrapulmonary, and intracardial exposure routes (Paul R. Badenoch et al., 1990; Cerva, 1967b; Culbertson et al., 1966). This analysis attempts to understand the risk associated with *Acanthamoeba* spp. presence in drinking water distribution systems, and as such only the intranasal and corneal data sets were modeled.

Acanthamoeba keratitis

Badenoch et al (1990) experimented with *Acanthamoeba* and *Corynebacterium* to study the effect of the pathogens in the corneas of female Porton rats. In this study, the authors inoculated the *Acanthamoeba* isolate Ac 118 (a group III isolate) and *Corynebacterium xerosis* into a short, peripheral incision of the cornea using a 10-µL microsyringe (Paul R. Badenoch et al., 1990). Amebae were grown in PYNFH medium and bacteria were grown in brain-heart infusion medium. Inocula were prepared with different combinations of *Acanthamoeba* Ac118 concentrations between 0 and 10^4 amebae and *C. xerosis* concentrations between 0 and 10^6 bacteria (P.R. Badenoch, Johnson, Christy, & Coster, 1991; Paul R. Badenoch et al., 1990). In previous experiments, there was no effect observed from graded doses of the group III isolate of *Acanthamoeba* or *C. xerosis* alone. Only injected together did suppurative keratitis occur (Paul

R. Badenoch et al., 1990). The endpoint of this study was infection. The data collected are shown in Table 4.1, as experiment numbers 1 and 2.

Central Nervous System Infections

Červa (1967) studied experimental animals (guinea pigs, mice, and rats) with the A1 strain of *Acanthamoeba* (*Hartmanella) castellanii* and different routes of infection. The three exposures studied were intranasal, intracardial, and intracerebral with death measured as the endpoint of response. The cultures of the amebae were grown in a medium with Proteose Peptone Difco as the base ingredient for several years and re-isolation of the amebae from the organs of infected animals was performed (Cerva, 1967a, 1967b). Experiment 3 in Table 4.1 represents the experiment in which white mice of the Czechoslovak H-strain weighing 13-15 grams were inoculated intranasally by placing 0.02 mL of fluid over the nares of the ethyl-ether anesthetized mice (Cerva, 1967b).

Culbertson et al. (1966) studied pathogenicity of the HN-3 strain of *A. castellanii* (Culbertson et al., 1966; Marciano-Cabral & Cabral, 2003). Cultures of amebae were grown in trypticase soy broth and diluted so that 0.03 mL of a concentrated suspension could be instilled intranasally into ether-anesthetized SPF mice by placing the fluid over the nares (Culbertson et al., 1966; Culbertson, Ensminger, & Overton, 1965; Culbertson, Holmes, & Overton, 1965). The responses recorded were death, brain invasion, Acute Meningoencephalitis (AME) or Granulomatous Amebic Encephalitis (GAE). The studies with a death and brain invasion response are represented in Table 4.1 by Experiment 4 and 5, respectively. The AME study is Experiment 6 and the GE study is Experiment 7.

Table 4.1: Dose Response Experiment Data for *Acanthamoeba* spp.

Experiment	Pathogen	Exposure	Endpoint	Dose*	Responses			Resource
					Positive	Negative	Total	
1	*Acanthamoeba* Ac 118 with 10^4 *C. xerosis*	Cornea	Infection	10	0	8	8	Badenoch et al. (1990)
				100	0	16	16	
				1000	2	16	18	
				10000	5	3	8	
2	*Acanthamoeba* Ac 118 with 10^6 *C. xerosis*	Cornea	Infection	10	0	8	8	Badenoch et al. (1990)
				100	0	16	16	
				1000	7	17	24	
				10000	10	0	10	
3	*Acanthamoeba castellanii*	Intranasal	Death	3	0	20	20	Cerva (1967b)
				30	2	18	20	
				300	1	19	20	
				3000	7	13	20	
				30000**	9**	11**	20**	
				300000	16	4	20	
4	*Acanthamoeba castellanii* HN-3	Intranasal	Death	100	2	78	80	Culbertson et al. (1966)
				500	4	76	80	
				1000	10	70	80	
5	*Acanthamoeba castellanii* HN-3	Intranasal	Brain Invasion	100	7	73	80	Culbertson et al. (1966)
				500	31	49	80	
				1000	43	37	80	
6	*Acanthamoeba castellanii* HN-3	Intranasal	AME	100	2	78	80	Culbertson et al. (1966)
				500	9	71	80	
				1000	21	59	80	
7	*Acanthamoeba castellanii* HN-3	Intranasal	GE	100	3	77	80	Culbertson et al. (1966)
				500	30	50	80	
				1000	27	53	80	

*Units for dose are number of organisms; ** The study had a discrepancy in the reported number and percentage of deaths for this dose group. It was assumed that the total subject size was the same as for the other five dose groups in the experiment and that the reported percentage was in error.

Analysis Methods

Maximum likelihood estimation was used to fit dose response models to the data sets identified in the literature as described in Haas et al. (2014). The data sets were selected based on certain criteria: a minimum of three doses had to be administered in the study and dose groups needed to include at least three animals. The studies also needed to describe the species of the

host, the strain of the pathogen, the doses administered, and the number of positive and negative responses to each dose group.

The exponential and the approximate form of the beta-Poisson were fit to the eight selected data sets using the statistical programming language, "R" (www.r-project.org) with previously developed computer code (Weir et al., 2017). Equation 1 is the exponential dose response model, where *P(d)* is the probability of response at dose *d* and *k* is the probability that a single organism can survive and initiate infection.

$$P(d) = 1 - e^{-kd} \tag{1}$$

The approximate form of the beta-Poisson dose response model is given by Equation (2) where *P(d)* is the probability of response at dose *d*, N_{50} is the median infective dose and α is a slope parameter (C. Haas et al., 2014). In the cases where death is the response, the N_{50} is the equivalent of the LD_{50}, the median lethal dose. The use of the approximate form of the beta-Poisson model was confirmed using the methodology outlined in Xie et al. (2017). The results of this analysis are available in Table S1 of the Supplementary Materials.

$$P(d) = 1 - \left[1 + \left(\frac{d}{N_{50}}\right) \cdot \left(2^{1/\alpha} - 1\right)\right]^{-\alpha} \tag{2}$$

Goodness of fit was determined by comparing the deviance of a fit to the critical χ^2 value at degrees of freedom equal to the number of doses minus the number of parameters and a 95% confidence. If both models were deemed a good fit to the data, the significance of improvement of fit between the two models was determined by comparing the difference in deviances with the

critical χ^2 value at 1 degree of freedom and a 95% confidence. Confidence intervals for the best-fit model were estimated via bootstrapping.

When multiple data sets were available for the same pathogen or route of exposure, a statistical pooling analysis was performed to ascertain whether the data set had the same underlying distributions. A likelihood ratio test was used to determine if data could be pooled.

RESULTS

Dose Response Model for *Acanthamoeba keratitis*

For Experiment 1, the exponential was the best-fit dose response model for the exposure to the cornea of rats with 10^4 *Corynebacterium* and increasing dosages of *Acanthamoeba* Ac118 strain. The minimized deviance of the exponential was 0.388, which was well within the χ^2 value at 3 degrees of freedom (7.81). The best fit exponential model had an LD_{50} of 6,886 amebae. The statistics of the model are summarized in Table 4.2 and the best-fit model with confidence bands and the *k* parameter histogram are shown in Figures 4.1 and 4.2, respectively.

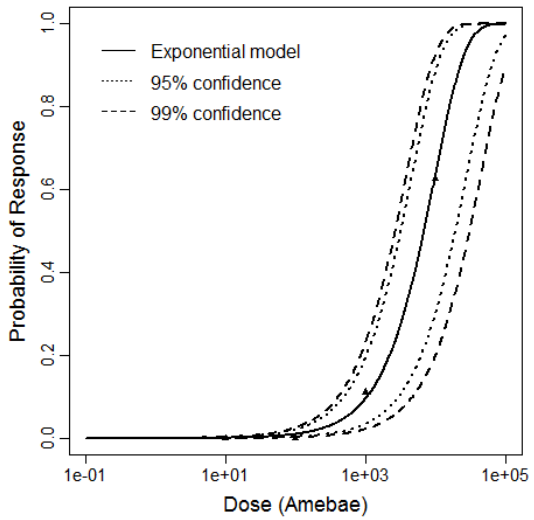


Figure 4.1: Plot of exponential model fit to Experiment 1 with upper and lower 95% and 99% confidence

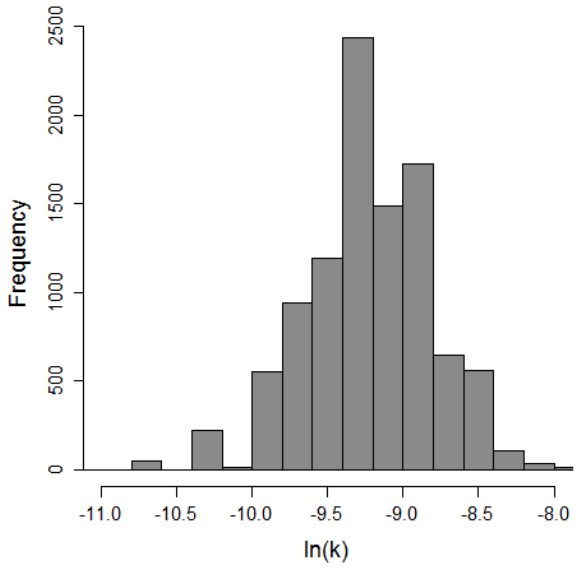


Figure 4.2: Uncertainty plot of exponential model for Experiment 1

For Experiment 2, the exponential was also the best-fit dose response model for exposure to the cornea of rat with 10^6 *Corynebacterium* and dosages of *Acanthamoeba* Ac118 strain. The minimized deviance of the exponential was 1.776, lower than the χ^2 value at 3 degrees of freedom (7.81). The best fit model had an LD_{50} of 1,907 amebae, indicating a higher virulence than Experiment 1. The statistics of the model are summarized in Table 4.2 and the best-fit model with confidence bands and the *k* parameter histogram are shown in Figures 4.3 and 4.4, respectively.

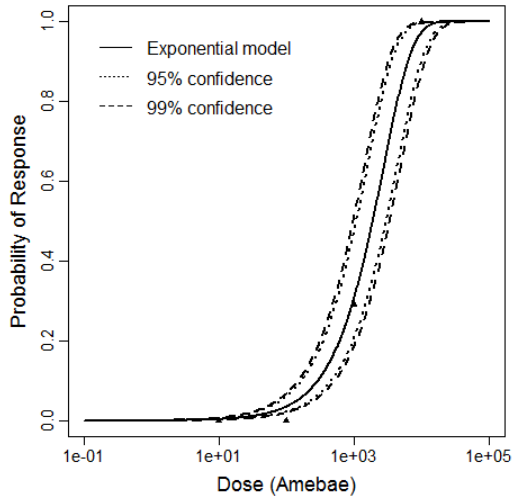


Figure 4.3: Plot of exponential model fit to Experiment 2 with upper and lower 95% and 99% confidence

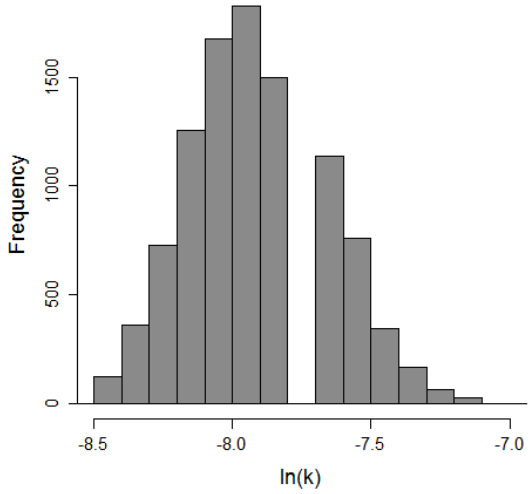


Figure 4.4: Uncertainty plot of exponential model for Experiment 2

Pooling analysis

Experiments 1 and 2 had different dosing groups of the same pathogens however a pooling attempt was unsuccessful. The difference between the sum of the individual best fits' deviances and the pooled best fit was 7.355, which is in excess of the critical distribution with 1 degree of freedom (3.84).

Dose-Response Model for Central Nervous System Infections

Death as an endpoint of response

The best-fit dose response model for mice exposed intranasally to *A. castellanii* in Experiment 3 was the approximate beta-Poisson. The minimized deviance of the approximate beta-Poisson was 6.83 and the χ^2 value at 4 degrees of freedom is 9.49. The exponential model did not provide an acceptable fit. The statistics for both models are in Table 4.2 and the best-fit

model with confidence bands is shown in Figure 4.5. The α and N_{50} cloud is shown in Figure 4.6 and represents the 90, 95, and 99% confidence of the parameters.

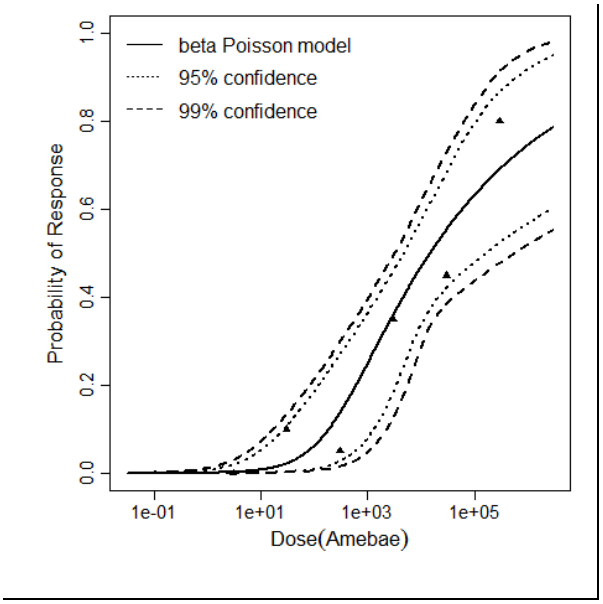
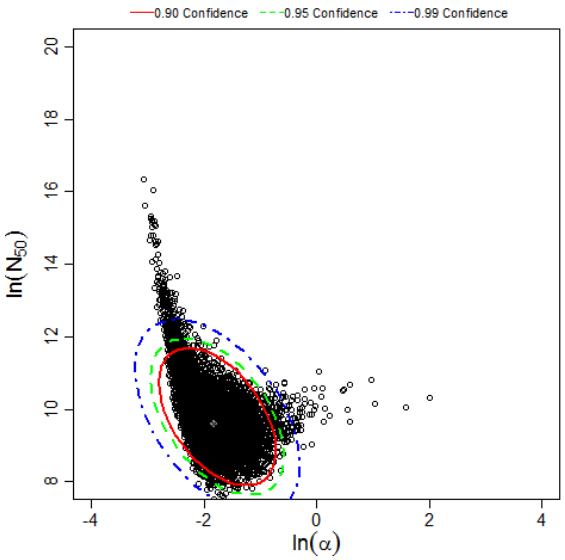


Figure 4.5: Plot of beta-Poisson Model fit to Experiment 3 with upper and lower 95% and 99% confidence



; **Figure 4.6:** Uncertainty plot of beta-Poisson Model for Experiment 3

For Experiment 4, the exponential model provided the best fit to the mice inoculated intranasally with *A. castellanii* HN-3 strain. The exponential had an LD_{50} of 5,276 amebae and

the statistics of the model are summarized in Table 4.2. The exponential model with confidence bands and the *k* parameter histogram are shown in Figures 4.7 and 4.8, respectively.

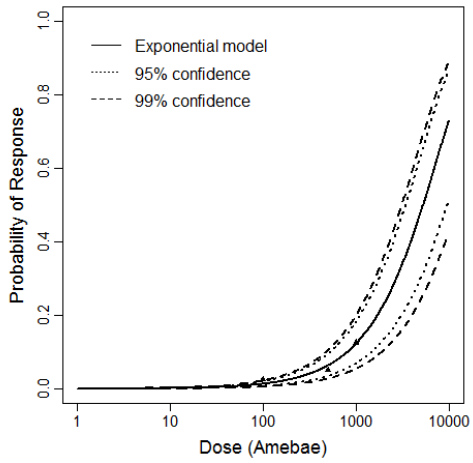


Figure 4.7: Plot of exponential model fit to Experiment 4 with upper and lower 95% and 99% confidence

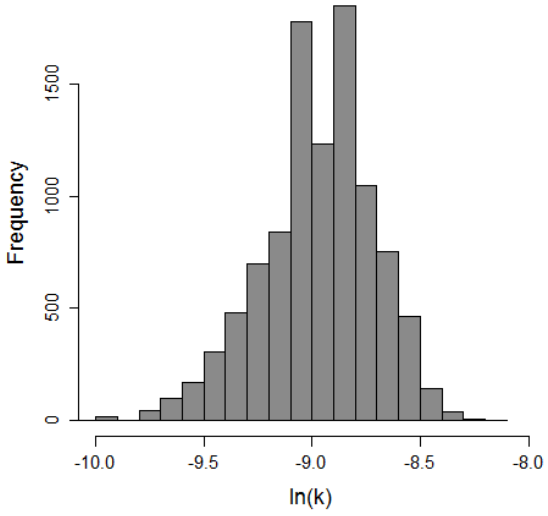


Figure 4.8: Uncertainty plot of exponential model for Experiment 4

Brain invasion as an endpoint of response

The exponential model was the best-fit dose response model to the mice inoculated intranasally with the *A. castellanii* HN-3 strain in Experiment 5. The ID_{50} for the brain invasion

model is 811 amebae, much lower than the LD_{50} for Experiment 5. The statistics of the model fits are summarized in Table 4.2 and the best-fit model with confidence bands is shown in Figure 4.9. The *k* parameter histogram is shown in Figure 4.10.

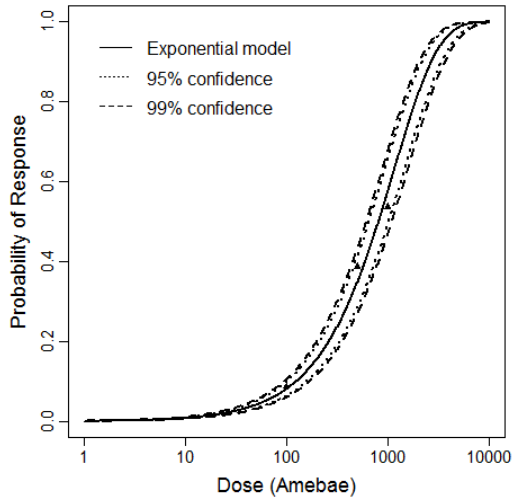


Figure 4.9: Plot of exponential model fit to Experiment 5 with upper and lower 95% and 99% confidence

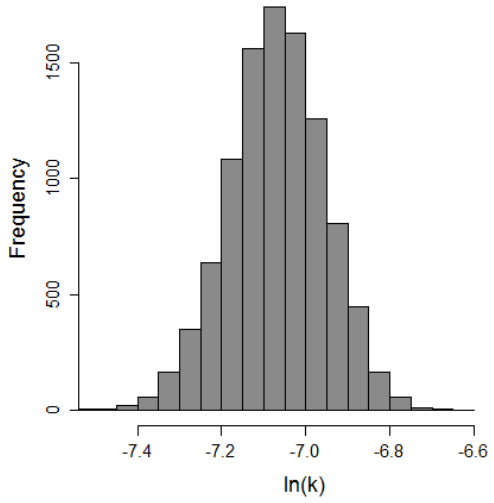


Figure 4.10: Uncertainty plot of exponential model for Experiment 5

Acute Meningoencephalitis (AME) as an endpoint of response

The exponential model provided best fit to the mice inoculated intranasally with *A. castellanii* HN-3 strain and the model's ID_{50} was 2,483 ameba. The minimum deviance for the exponential model was 0.405 and the χ^2 value at 2 degrees of freedom is 5.99. The statistics of the model fits are summarized in Table 4.2 and the best-fit model with confidence bands is shown in Figure 4.11. The k parameter histogram is shown in Figure 4.12.

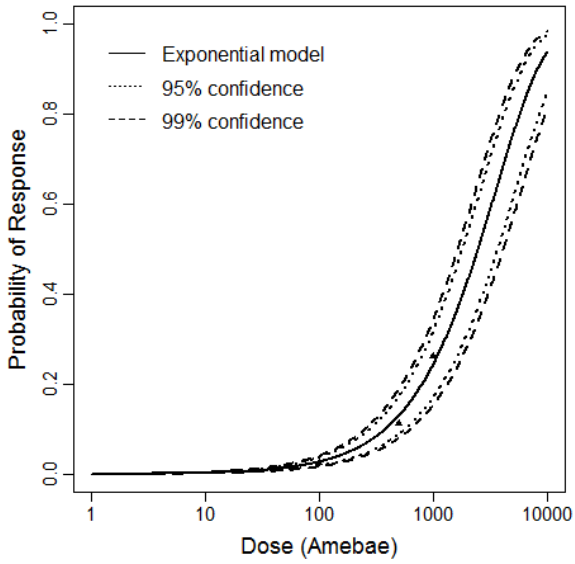
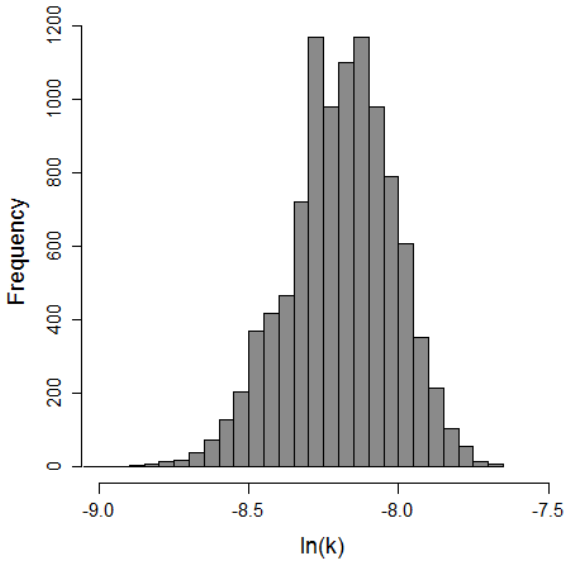


Figure 4.11: Plot of exponential model fit to Experiment 6 with upper and lower 95% and 99% confidence



4.12: Uncertainty plot of exponential model for Experiment 6

GAE as an endpoint of response

Neither model fit the data for the mice inoculated intranasally with *A. castellanii* HN-3 strain and GE as the response. The minimum deviance for the exponential model was 10.0 and 7.574 for the exponential and approximate beta-Poisson, respectively, which was larger than the χ^2 values at their respective degrees of freedom. The data used for Experiment 7 is shown in Table 4.1.

Table 4.2: Statistics for *Acanthamoeba* spp. Dose Response Models

Experiment	Model	Deviance	Δ	DF	$\chi^2_{0.95,df}$	$\chi^2_{0.95,1}$	Best Fit	Parameters	LD50/N50
1	Exponential	0.388	0.0077	3	7.81	3.84	Exponential	k=1.01E-04	6886
	beta-Poisson	0.381		2	5.99				
2	Exponential	1.776	0.0002	3	7.81	3.84	Exponential	k=3.63E-04	1907
	beta-Poisson	1.776		2	5.99				
3	Exponential	55.6	48.77	5	11.1	3.84	beta-Poisson	α=1.61E-1 N50=14538	14538
	beta-Poisson	6.83		4	9.49				
4	Exponential	0.969	0.102	2	5.99	3.84	Exponential	k=1.31E-04	5276
	beta-Poisson	0.864		1	3.84				
5	Exponential	1.0275	0.7052	2	5.99	3.84	Exponential	k=8.54E-04	811.4
	beta-Poisson	0.3223		1	3.84				
6	Exponential	0.4049	0.0019	2	5.99	3.84	Exponential	k=2.79E-04	2483
	beta-Poisson	0.4069		1	3.84				

Pooling analysis

A pooling analysis was attempted for the experiments 4, 5, and 6 because they all employed the same pathogen, the *A. castellanii* HN-3 strain. A minimized deviance of 85.65 and 84.60 for the exponential and approximate beta-Poisson models, respectively, was well above their respective χ^2 values of 15.50 and 14.07. Thus, the data could not be pooled.

Experiment 3 dealt with an intranasal inoculation of *A. castellani* with death as the endpoint response. The same exposure route and endpoint were measured for the *A. castellanii* HN-3 in experiment 4 and thus a pooling analysis was attempted. The exponential model did not show a good fit to the data, however the approximate beta-Poisson model did show a good fit.

The minimized deviance was 11.04 and the χ^2 value is 14.07. The difference between the sum of the individual best fits' deviances and the pooled best fit was 3.241, which is less than the critical value at 1 degree of freedom (3.84). The parameters from the pooled model are shown in Table 4.3. Figure 4.13 is the beta-Poisson model with confidence bands, and Figure 4.14 shows the plot of the 90%, 95%, and 99% confidence values for the parameters.

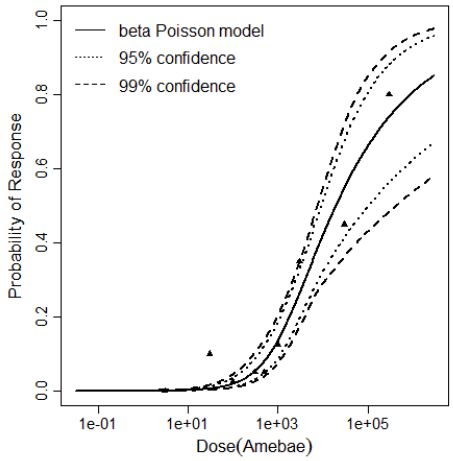


Figure 4.13: Plot of beta Poisson model fit to the pooled Experiments 3 and 4 with upper and lower 95% and 99% confidence

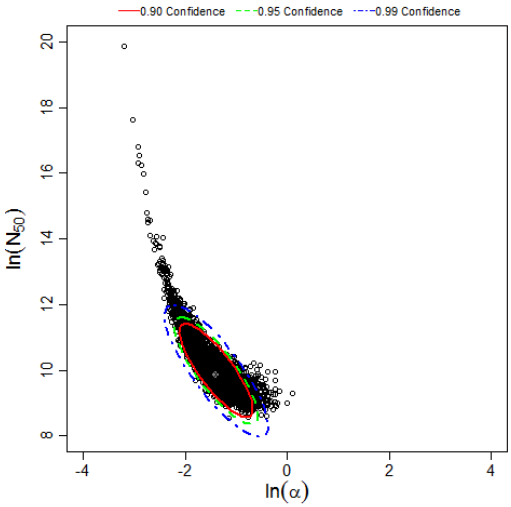


Figure 4.14: Uncertainty plot of beta Poisson model for the pooled Experiments 3 and 4

Table 4.3: *A. castellani* Pooling Statistics

Data Set	Number of Doses	Best fit Model	Minimized Deviance	D.O.F.	$\chi 2\alpha,n-k$	$\chi 2$ p-value	Parameters
Pooling 3 and 4	9	beta-Poisson	11.04	7	14.07	0.1367	α=0.245014 N_{50}=19348

DISCUSSION

Acanthamoeba keratitis was only observed when *Acanthamoeba* Ac 118 was inoculated into the cornea in combination with high doses of *C. xerosis*. As the amount of *Corynebacterium* increased from 10^4 to 10^6, the fit of the curve became steeper, indicating a greater level of virulence. This conclusion is echoed by the ID_{50} value. Badenoch et al. (1990) added the *C. xerosis* into the inoculum when neither organism alone could induce the production of an infiltrate (P.R. Badenoch et al., 1991). This suggested that the *Corynebacterium* somehow allowed *Acanthamoeba* to survive and initiate infection in the cornea (Paul R. Badenoch et al., 1990). Seventeen other *Acanthamoeba* isolates were investigated with an inoculum of 10^6 *Corynebacterium* and five of the isolates were able to induce suppurative keratitis (P.R. Badenoch et al., 1991). However other animal models of *Acanthamoeba* keratitis have not shown a dependence on a coinfection with a bacterial strain (Marciano-Cabral & Cabral, 2003). It is likely that this dependence is strain and host dependent. The Ac 118 strain of *Acanthamoeba* was isolated from a GAE infection (Paul R. Badenoch et al., 1990). It is possible that when introduced to the eye this isolate of *Acanthamoeba* requires a symbiotic relationship with another species that is part of the natural flora or that is pathogenic to the eye. Understanding potential relationships with bacteria that facilitate infections by different strains of *Acanthamoeba* is valuable because some bacteria such as *Corynebacterium* spp. are part of the normal flora for human and animals, and there are also other bacterial species present in drinking water that could interact with the amebae (Vela, Gracía, Fernández, Domínguez, & Fernández-Garayzábal, 2006).

It should be noted, however, that other dose response models need to be developed for species of *Acanthamoeba* known to independently cause keratitis to provide the most accurate estimations of the risk that *Acanthamoeba* poses to tap water users.

Cerva (1967b) and Culbertson et al. (1966) studied death in mice after intranasal exposures to the A1 strain and HN-3 strain of *A. castellanii*, respectively. The HN-3 strain had a much lower LD_{50} than the experiment conducted by Cerva (1967b), suggesting that the HN-3 strain may have a higher virulence. Cerva (1967b) determined that the growth phase of the amebae had a large influence on virulence and it is possible that these separate experiments had inoculum with amebae in different growth stages, explaining the LD_{50} discrepancy. However, both of these experiments could be successfully pooled suggesting the strains are mechanistically similar, and the pooled model had the highest LD_{50} of all with a value of 19,348 amebae. By adjusting the growth phase of the amebae, Cerva (1967b) could vary the LD_{50} value from 300 to 300,000 amebae in the inoculum. This pooled model likely encompasses that variation and as such has a higher LD_{50} than the individual models. The intranasal route of exposure is important to model for *Acanthamoeba,* as it is thought that the inhalation of amebae may be the portal of entry for GAE infections (Ma et al., 1990; Marciano-Cabral & Cabral, 2003). *Naegleria fowleri*, another FLA capable of causing a central nervous system infection that can lead to death, has an LD_{50} of 13,257 amebae for the intranasal route of exposure, which is approximately 6,000 amebae lower than the LD_{50} reported in this study for *Acanthamoeba*. As it is rarer for *Acanthamoeba* to cause CNS infections, and *N. fowleri* is known to cause a highly fatal infection, Primary Amebic Meningoencephalitis, this discrepancy makes sense (Dean, Weir, et al., 2019). Of all the experiments modeled in this study, the intranasal route of exposure with brain invasion as an endpoint of response had the lowest ID_{50}, with a value of 811 amebae. This

endpoint of response was the least severe of the four studied (brain invasion, AME, GAE, and death) and it is logical that it had the lowest median infectious dose.

The corneal and intranasal exposure routes are addressed in this study because of the presence of the FLA in drinking water. However in a previous investigation of an *Acanthamoeba* keratitis outbreak in the USA, water exposures such as showering, bathing, or swimming were not statistically significant risk factors (Yoder et al., 2012). Another study done in the UK, where the prevalence of *Acanthamoeba* keratitis is higher, sampled taps from the homes of 27 people with confirmed cases of *Acanthamoeba* keratitis and FLA were identified in 24 of the 27 households (Simon Kilvington et al., 2004; Shoff, Rogerson, Kessler, Schatz, & Seal, 2008). *Acanthamoeba* spp. specifically was identified in 30% of the homes (Simon Kilvington et al., 2004). *Acanthamoeba* spp. was also detected in 6.7% of samples (n=90) from a drinking water distribution system in southwest Virginia. The positive samples had an average concentration of 2.2 gene copies/mL +/- 2.4 gene copies (Wang, Edwards, Falkinham, & Pruden, 2012). Despite its presence on the first Contaminant Candidate List (CCL) published by the Environmental Protection Agency, it has not been on a CCL since, suggesting that it has not been prioritized (Gerba et al., 2003; US Environmental Protection Agency, 1998). In addition to being the etiologic agent for eye and CNS infections, *Acanthamoeba* spp. is also known to facilitate the proliferation of bacterial species such as *Legionella* spp. and the *Mycobacterium avium* complex, further making it a drinking water concern (Hamilton, Weir, et al., 2017; Lau & Ashbolt, 2009). The dose response models developed in this analysis will help facilitate QMRAs that will further elucidate the potential risk posed by *Acanthamoeba* spp.

CONCLUSIONS

This work contributes to the growing focus on FLA, opportunistic pathogens and the human health risk they pose in environments like drinking water distribution systems. *Acanthamoeba* spp. are capable of causing eye and central nervous systems infections, and dose response models were developed in this study for multiple exposure routes and endpoints of response. Understanding the risk of a health endpoint per given exposure dose will allow for more effective control and maintenance of environments where exposure is possible. A person is at a different level of risk when they are ingesting, inhaling, or being dermally exposed to the possibly contaminated water. Developing dose response models for these scenarios is one of the first steps needed to help decision makers characterize and manage the risk.

CHAPTER 5: A DOSE RESPONSE MODEL FOR THE INHALATION ROUTE OF EXPOSURE TO *P. AERUGINOSA*

INTRODUCTION

P. aeruginosa is a gram-negative bacterium associated with respiratory infections. It is a common cause of nosocomial, ventilator-associated, and community-acquired pneumonia, and immunocompromised hosts and patients with cystic fibrosis are at a higher risk of infection (Driscoll, Brody, & Kollef, 2007a; Sadikot, Blackwell, Christman, & Prince, 2005). Although less common, *P. aeruginosa* infection in healthy individuals has a reported mortality of 33% (Hatchette, Gupta, & Marrie, 2000; Sadikot et al., 2005). A study analyzing patients admitted to the Respiratory Intensive Care Unit in the Hospital Clinic of Barcelona observed a similar mortality rate and determined that *P. aeruginosa* was the only etiologic agent significantly associated with mortality in patients with community-acquired pneumonia (Torres et al., 1991).

P. aeruginosa can exist in a range of environments. In healthcare settings, water related sites like taps and showers and moist, humid environments like respiratory therapy equipment are the most likely to be colonized (Kerr & Snelling, 2009). *P. aeruginosa* develops biofilms that aid in its production of virulence factors and its persistence in its environment and biofilms in these water systems can become an ideal long-term habitat for this opportunistic pathogen (Sadikot et al., 2005; Wingender & Flemming, 2011a). Although it is known that *P. aeruginosa* colonizes premise plumbing systems and point-of-use devices like showerheads and faucets, the threat to the user is uncertain. It is possible that during a showering event the pathogen may be aerosolized and inhaled. In order to be able to understand the risk of pneumonia from *P. aeruginosa* in such an exposure scenario, it is first necessary to have an understanding of the dose response relationship.

A dose response model for the inhalation route of exposure of *P. aeruginosa* has not previously been developed but would be valuable in facilitating future risk assessments and help determine if there is a need for remediation of premise plumbing. This study aims to fit dose response models to pre-existing data. Such models could be used to provide a greater understanding of the threat posed by opportunistic pathogens like *P. aeruginosa* in premise plumbing systems is needed to protect human health.

METHODS

A review of the literature was conducted to find a dose response study that simulated an inhalation exposure, had three or more dosing groups, and documented the positive and negative responses from each dose. A study conducted by Ojielo et al. (2003) evaluated the risk of pulmonary infection after bone marrow transplantation in mice. The researchers first evaluated the course of *P. aeruginosa* pneumonia in normal hosts by intratracheally inoculating groups of 10 wild-type, specific pathogen-free B6D2F1/J mice with six varying doses of *P. aeruginosa*. *P. aeruginosa* PAO1 frozen stock was grown in 10 mL of tryptic soy broth at 37°C. The trachea was exposed in a sterile fashion and a 26-gauge needle was used to administer the inoculum intratracheally. The positive endpoint response was death (Ojielo et al., 2003). In addition to these trials, the researchers intratracheally inoculated a group of 7 wild-type mice with a dose of 2×10^6 CFU to compare the survival rates to those of the bone marrow transplantation recipient mice. The dose of 2×10^6 CFU for the wild-type mice was included in this analysis because the exposure route, pathogen strain, host and endpoint were the same as for the previous six doses (Ojielo et al., 2003). The seven dosing groups and the respective responses are shown in Table 5.1.

Table 5.1: Dose Response Data for *P. aeruginosa* from Ojielo et al. (2003)

Dose (CFU)	Positive Response	Negative Response
80,000	0	10
400,000	0	10
900,000	0	10
2,000,000	2	5
3,000,000	6	4
4,500,000	10	0
8,000,000	10	0

Using the statistical programming language, "R", and a previously developed code that uses maximum likelihood estimation (MLE) methods as outlined in Haas et al. (2014) (Weir et al., 2017), the exponential and beta-Poisson models were fit to the dose response data. The exponential model (Equation 1) determines the probability of a response, *P(d)*, based on the dose, *d*, and the parameter, *k*, which represents the likelihood that a single organism survives to initiate infection.

$$P(d) = 1 - e^{-kd} \qquad \text{Eq. 1}$$

The approximate form of the beta-Poisson model (Equation 2) determines the probability of response based on the α parameter, which dictates scale, and the median infective dose, N_{50}. The endpoint of response for the dose response data being considered is death, and as such the N_{50} and LD_{50} are equivalent.

$$P(d) = 1 - [1 + \left(\frac{d}{N_{50}}\right) * \left(2^{1/\alpha} - 1\right)]^{-\alpha} \qquad \text{Eq. 2}$$

Both the exponential and beta-Poisson models are "single-hit" models; they operate under the assumption that just a single organism, k_{min} equal to 1, is needed to initiate infection.

The cooperativity theory assumes that for some pathogens, a k_{min} greater than 1 may be necessary to initiate infection (C. Haas et al., 2014). This different assumption results in the multi-hit dose response model (Equation 3). The multi-hit dose response model is represented by the incomplete gamma function (C. Haas et al., 2014). As it follows the gamma probability distribution, the multi-hit model can be coded in R using the ***pgamma()*** function from the stats package. It is coded as ***pgamma(x, a)***, where ***x*** is the dose, *d*, multiplied by the probability that the pathogen survives to initiate infection, *k*. The k_{min} value is ***a***. Note, when k_{min} is equal to one, the probabilities output by the multi-hit model are equivalent to that of the exponential model.

$$P(d) = \Gamma(k_{min}, d * k) \qquad \text{Eq. 3}$$

The multi-hit model was fit iteratively, with the k_{min} parameter fixed at values ranging from 1-187 and the *k* parameter determined using MLE methods as described above. Both parameters could not be solved for using MLE methods simultaneously because they are inherently correlated and the k_{min} value should be an integer, as it represents a number of pathogens.

Goodness of fit was determined by comparing the optimized deviance of the model to the χ^2 distribution with the degrees of freedom equal to the number of parameters of the model subtracted from the number of doses (C. Haas et al., 2014). The null hypothesis, that the model provides an acceptable fit, is rejected if the deviance value exceeds the critical χ^2 value. The best fitting model was determined by comparing the difference in deviances between the models to the critical χ^2 value at one degree of freedom. Confidence bands were determined by bootstrapping the data.

RESULTS

The exponential model fit the dose response data with a deviance of 16.88, which is greater than the χ^2 value at 5 degrees of freedom (11.1). The beta-Poisson model had a deviance of 16.88 compared to the critical χ^2 value of 9.49 for 4 degrees of freedom. The exponential model was a better fit than the beta-Poisson, however neither model was a statistically good fit. The fit statistics for both models are shown in Table 5.2.

Table 5.2: Conventional Dose Response Model Fit Statistics for *P. aeruginosa*

Model	Deviance	Δ	DF	$\chi^2_{0.95,df}$	$\chi^2_{0.95,1}$	Best Fit	Parameters	LD50
Exponential	16.88	0.001	5	11.1	3.84	Exponential	k= 3.22E-07	2,150,065
beta-Poisson	16.88		4	9.49				

The multi-hit dose response model fit the data with a deviance of 1.09, which is below the critical χ^2 value of 11.1. The parameters for the best fitting model were a *k* value of 4.12E-06 and a k_{min} of 11. The fit statistics are shown in Table 4.3. The multi-hit model is depicted in Figure 5.1 with 95 and 99% confidence bands. Figure 5.2 is a histogram of the *k* parameter estimates after bootstrapping. The model was fit for k_{min} values ranging from 1 to 187 to ensure the optimal fit statistics were identified. After a k_{min} of 187, the model was no longer able to find an optimum *k* value. Figure 5.3 illustrates the minimum deviance value for each iteration with the lowest deviance and respective k_{min} value identified in red.

Table 5.3: Multi-hit Dose Response Model Fit Statistics for *P. aeruginosa*

Model	Deviance	Δ	DF	$\chi^2_{0.95,df}$	$\chi^2_{0.95,1}$	Best Fit	Parameters	LD50
Multi-hit	1.09	15.69	4	9.49	3.84	Multihit	*k*= 4.12E-06 k_{min}=11	2,588,047

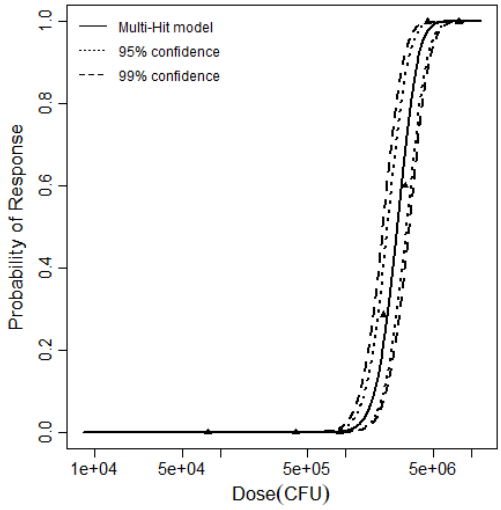


Figure 5.1: The multi-hit dose response models with 95 and 99% confidence bands

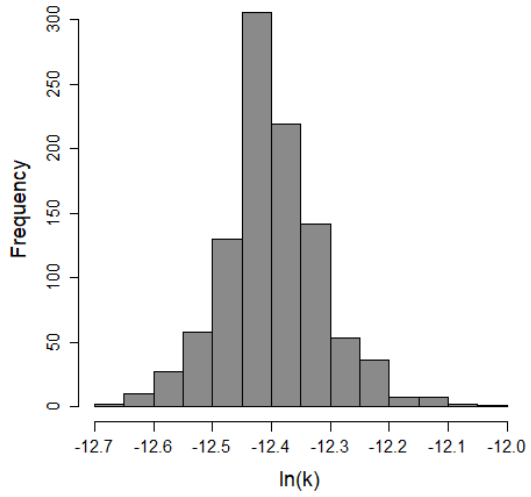


Figure 5.2: Histogram of the k parameter estimates after bootstrapping

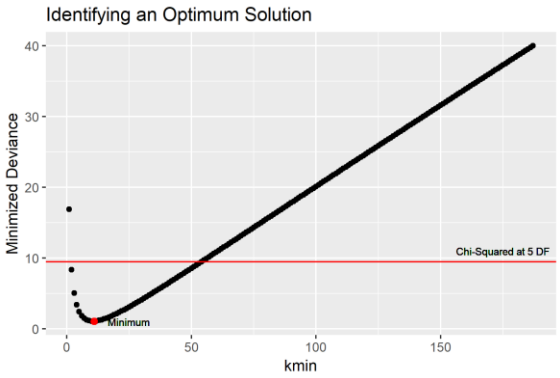


Figure 5.3: The k_{min} value and minimum deviance from each iteration of fitting the multi-hit model, illustrating the optimal solution at k_{min} =11

DISCUSSION

This study fit the multi-hit dose response equation to dose response data for *P. aeruginosa* after neither the exponential or beta-Poisson model provided a statistically significant fit. The multi-hit model has not been traditionally applied to microbial data but has been used in toxic chemical risk assessment (Janardan, 1986). The slope of the multi-hit model is greater than that of the exponential at the median infectious dose and most microbial experimental data show slopes equal to or less than the exponential model (C. Haas et al., 2014). One-hit models are based on the hypothesis of independent action; a hypothesis that states that pathogenic individuals behave independently of one another and each have an independent probability of causing infection or death (Cornforth, Matthews, Brown, & Raymond, 2015; Druett, 1952). The statement of independent action equates to a k_{min} value equal to 1. Single-hit models are not threshold models and assume low dose linearity. The multi-hit model assumes a k_{min} >1, does not have low dose linearity, and is a simple threshold model. Historically there has been stronger evidence for the biological plausibility of the one-hit model than for the multi-hit (C. Haas et al., 2014).

However research into molecular mechanisms involved in infection have demonstrated that several cooperative behaviors within pathogens help facilitate the evasion of host defenses and initiate infection. There is concern that single-hit theory models can not capture these behaviors and host-pathogen interactions, making the models overly conservative (Coleman et al., 2017). It was demonstrated that the independent action theory failed in *Bacillus thuringiensis*, an insect pathogen, because of the cooperative nature of the bacteria's toxins. Cell cooperation is necessary for *B. thuringiensis* to facilitate host invasion and septicaemic proliferation (Cornforth et al., 2015). A study of *Bacillus anthracis* demonstrated that disruption of the pathogen's quorum sensing inhibited growth and virulence gene expression in vitro, suggesting that a single spore would not result in growth, disease progression, or mortality (Coleman, Thran, Morse, Hugh-jones, & Massulik, 2008; Jones, Jani, Ren, Wood, & Blaser, 2005). The need to analyze both threshold and non-threshold dose response models for *B. anthracis* was suggested in response to the restrictions overestimated low dose risks places on risk management solutions (Coleman et al., 2008).

These examples of failings of the independent action hypothesis adds credence to the application of a simple threshold model to the *P. aeruginosa* dose response data. Similar to the previously mentioned pathogens, there are cooperative action indicators in the pathology of *P. aeruginosa*. *P. aeruginosa* rarely infects the lungs of an immunocompetent host, despite its ubiquitous nature. Some of the bacterial factors involved in the pathogenesis of *P. aeruginosa* lung infections include pili and flagella, a Type III secretion system, and quorum sensing (Sadikot et al., 2005). Data suggests that *P. aeruginosa* needs to express several virulence factors to initiate a pulmonary infection and the outcome of the infection is dependent on the host response, which in the lung environment can be quite robust (Cohen & Prince, 2013; Tang et al.,

1996). Quorum sensing promotes biofilm formation which enables the pathogen to evade host defenses and persist in the environment (Sadikot et al., 2005; Wu et al., 2004). Furthermore, quorum-sensing in *P. aeruginosa* regulates expression of extracellular virulence factors (Pearson, Feldman, & Iglewski, 2000; M. Schuster, Sexton, Diggle, & Greenberg, 2013). Targeting the quorum-sensing system of *P. aeruginosa* is being explored as an alternative to antibiotic treatment and a study analyzing the quorum-quenching effects of lactonases reduced *P. aeruginosa* pneumonia mortality in rats from 75% to 20% (Hraiech et al., 2014).

The reliance on cell-to-cell communication suggests that a single bacterium may not be enough to initiate infection. A greater understanding of cooperative behavior in microorganisms indicates that dose response modeling of microbes may not be limited to only the single-hit theory models traditionally used. This study fit a simple threshold model for an inhalation exposure to *P. aeruginosa* with an LD_{50} of 2,588,047 CFU. Neither of the single-hit dose response models provided significant fits to the data. The multi-hit model indicates survivability below a certain point, roughly 500,000 CFU, and then a rapid increase in mortality with dose. Compared to the exponential model, the multi-hit model estimates much lower probabilities of death at low doses. Although more conservative estimates have often been preferred, it has more recently been suggested that QMRA models often overestimate risk, likely due to overly conservative dose response models (Coleman et al., 2017; Snary et al., 2016).

CONCLUSIONS

The dose response model created in this study is the first dose response model for the inhalation route of exposure for *P. aeruginosa*. The multi-hit model provided a significant fit to the data and indicates that perhaps microbial dose response modeling should not be limited to single-hit theory models. A dose response model was needed for this route of exposure to

facilitate the completion of quantitative microbial risk assessments to address exposure scenarios of concern. Possible exposure scenarios may include inhaling aerosols in a showering event, through using a humidifier, or in pools and hot tubs. This model and future QMRA models will aid risk managers and decision makers about how best to treat water to protect against opportunistic pathogens, including *P. aeruginosa*.

CHAPTER 6: REVERSE QMRA OF *P. AERUGINOSA* IN PREMISE PLUMBING TO INFORM RISK

INTRODUCTION

Pseudomonas aeruginosa is an opportunistic pathogen that poses a significant threat to the immunocompromised population. *P. aeruginosa* causes community-acquired and hospital-acquired infections, including folliculitis, keratitis, bacteremia, soft tissue and wound infections, urinary tract infections and pneumonia (Driscoll et al., 2007a; Kerr & Snelling, 2009). It has been identified as the second most frequent cause of hospital-acquired, healthcare-associated, and ventilator-associated pneumonia (Driscoll et al., 2007a; Joseph O. Falkinham, 2015; Sadikot et al., 2005). For immunocompromised hosts, *P. aeruginosa* is considered the most important pathogen in patients with primary and acquired immunodeficiencies (Driscoll et al., 2007a). Patients with cystic fibrosis are especially susceptible, and *P. aeruginosa* is the leading cause of pneumonia, causing chronic lung infection and an increase in morbidity and mortality rates (Streeter & Katouli, 2016). The bacterium is also the leading cause of bacterial keratitis, affecting individuals after eye surgery, people with ocular disease and contact lens wearers (Streeter & Katouli, 2016).

The types of infections caused by *P. aeruginosa* are diverse and its ubiquitous nature makes its management a clear concern for hospitals and communities alike. Sources of known exposure include hot tubs, swimming pools, colonized medical equipment, and tap water. The bacterium thrives in moist environments and it commonly lives and grows in biofilms in plumbing systems (Trautmann et al., 2005). The biofilm allows the bacterium to be more resistant to disinfectants, antibiotics, and other antagonizing factors (Bédard et al., 2016; Moritz, Flemming, & Wingender, 2010). The presence of *P. aeruginosa* in tap water has been strongly

associated with the colonization of the faucets, drains, sinks, and showerheads (Bédard et al., 2016; Trautmann et al., 2005). The colonization of these point-of-use fixtures facilitates exposure through several different routes. Aerosolization from the fixture-head allows for an inhalation exposure that could result in a respiratory infection. Direct application of contaminated water on the skin or eyes could result in dermal and ocular infections.

Quantitative microbial risk assessment (QMRA) is used to estimate the risk of infection from exposure to microorganisms in diverse environments (C. Haas et al., 2014). It is a widely accepted framework for water safety guidelines to support public health. QMRA is used to establish design criteria for treatment plants and to establish monitoring plans (National Research Council, 2006). Currently, *P. aeruginosa* is not on the EPA's Contaminant Candidate Lists and it is not currently monitored or regulated (US Environmental Protection Agency, 1998, 2005, 2009, 2016). However, it is important for water managers and building operators to know what concentrations of *P. aeruginosa* might warrant immediate action and management when detected in tap water. Though treatment guidelines for pathogens in water are generally based on the ingestion route of exposure, the concentration level of concern may likely vary based on the exposure pathway of concern.

This study aims to determine the threshold concentrations of *P. aeruginosa* in tap water that warrant risk management using a reverse QMRA framework for the two most relevant exposure routes under three different scenarios: a showering, face washing, and hand washing event. The conclusions drawn from these reverse QMRAs will determine threshold levels of *P. aeruginosa* that should be monitored for and controlled. Applying this type of modeling to engineered water systems can help identify pathogens of priority and help risk managers properly allocate funds for monitoring, sampling, and treatment procedures.

METHODS

QMRA Framework

A reverse QMRA takes an accepted risk threshold and calculates the quantity of pathogens in the water that would cause this level of risk across a specific exposure pathway. Such a modeling approach was previously described and published for a showering exposure scenario that predicted *Legionella* densities of concern in shower air, water, and in-premise plumbing biofilms associated with target deposited doses of *Legionella* in the alveolar region (Schoen & Ashbolt, 2011). This study utilizes the U.S. EPA's maximum allowable risk for microbial contaminants in water of 1 infection per 10,000 persons per year (Macler & Regli, 1993; O'Toole, Sinclair, Gibney, & Leder, 2015). The motivation for this risk assessment is to inform future monitoring and sampling protocols for managers of drinking water distribution networks. Thus, the risk threshold of 1 infection in 10,000 persons per year is transformed into an average daily risk to give water and building managers the information needed to protect the health of their consumers. To transform the average yearly risk, $P(d)_{annual}$, into a daily risk, $P(d)_{daily}$, Equation 1 was used with the assumption that the exposures occur each day of the year.

$$P(d)_{annual} = 1 - (1 - P(d)_{daily})^{365} \quad \text{Eq. 1}$$

For each exposure, a dose response model was used to calculate the exposure dose associated with the average daily risk of infection. A dose response model is a mathematical function that conveys the relationship between the microbial exposure or dose and the likelihood of occurrence of an adverse effect such as infection, illness, or death (C. Haas et al., 2014). Two of the most commonly fit dose response models are the exponential and beta-Poisson. Equation 1 is the exponential model, where $P(d)$ is the probability of response at dose d, and the single parameter k represents the probability that a single organism survives to initiate the observed

response. Equation 2 is the approximate form of the beta-Poisson dose response model. The two parameters are the N_{50}, which represents the dose at which 50% of the exposed population succumbs to the adverse health effect (infection, illness or death), and the shape parameter α (C. Haas et al., 2014). The concentration in the bulk water that would result in this exposure dose was then determined through a detailed exposure assessment described below.

$$P(d) = 1 - e^{-kd} \quad \text{Eq. 2}$$

$$P(d) = 1 - [1 + \left(\frac{d}{N_{50}}\right) \times \left(2^{1/\alpha} - 1\right)]^{-\alpha} \quad \text{Eq. 3}$$

Dose Response

Inhalation Dose Response Model

An inhalation dose response model with death as an endpoint response was recently developed, and the multi-hit dose response model (Equation 4) from that analysis was used in this study (Dean, 2019). The multi-hit dose response model has parameters: *k* equal to 4.12E-06; k_{min} equal to 11; and N_{50} equal to 2,588,047 CFU, where N_{50} corresponds to LD_{50} for the lethal endpoint. In order to use this dose response model to estimate the dose corresponding to the EPA's standard of 1 infection in 10,000 persons, it was necessary to apply a morbidity rate, the probability of illness given infection, and a mortality rate, the probability of death given illness. The morbidity rate was assumed to be 100%. The mortality rate for community-acquired pneumonia due to *P. aeruginosa* was assumed to be the same for *P. aeruginosa* infection in previously healthy individuals that were exposed to heavily contaminated aerosols-a rate of 33% (Sadikot et al., 2005). Based on these assumptions, the risk of infection of 1 in 10,000 corresponded to an estimated risk of 3.33 deaths per 100,000 persons annually via the inhalation

route of exposure. Due to the continuously varying nature of the multi-hit dose response function, shown in Equation 4, the exposure dose was calculated using integration techniques.

$$P(d) = \Gamma(k_{min}, d * k) \quad \text{Eq. 4}$$

Corneal Dose Response Model

Previously published dose response models for the ocular route of exposure were available on the QMRA wiki (Tamrakar, 2013). The recommended dose response model was developed by fitting the dose response data from a study using *P. aeruginosa* contaminated contact lenses to inoculate the eyes of New Zealand rabbits in order to produce keratitis as the health endpoint. The best fitting model was a beta-Poisson with an α of 0.19 and an N_{50} of 18,500 CFU. This was the model selected and applied in this analysis because the exposure route was relevant to the pathways explored and the endpoint measured corresponds to infection as the response. The daily risk of infection, *P(d)*, that was calculated from Equation 1 was directly substituted into Equation 5 to determine E_D.

$$E_D = [(1 - P(d))^{-1/\alpha} - 1]\frac{N50}{2^{1/\alpha} - 1} \quad \text{Eq. 5}$$

Exposure Assessment

To determine the concentrations in the water responsible for the calculated exposure doses, detailed exposure assessments were constructed for a showering, face washing, and hand washing event in a typical residence setting. The showering exposure models an average adult over the age of 21 that showers once a day for a year. The face washing exposure assesses the risk of the average adult washing their face with their eyes partially open, once a day for a year. Finally, the hand washing event specifically addresses the scenario when the average adult washes their hands and afterwards inserts or removes their contacts, two times a day for a year.

Showering Event

The inhalation exposure in a showering event was described by Equation 6. Exposure dose (E_D) is a function of the concentration in the water (C_w) in CFU/L multiplied by a partitioning coefficient (*PC*) in L/m^3 to estimate the concentration of pathogens aerosolized. The quantity of pathogens in the lungs to initiate infection is determined by the inhalation rate (*IR*) in m^3/minute, time of the showering event (*T*) in minutes, the fraction of aerosols of a respirable size (F_{RA}), and the retention rate (*RR*) (i.e. the percent deposited in the alveolar region). To accommodate this QMRA in reverse, these variables are rearranged into Equation 7 in order to calculate *Cw*, the concentration in the bulk water responsible for a risk level of 1 infection in 10,000 persons.

$$E_D = C_w \times PC \times IR \times T \times F_{RA} \times RR \quad \text{Eq. 6}$$

$$C_w = \frac{E_D}{PC \times IR \times T \times F_{RA} \times RR} \quad \text{Eq. 7}$$

Face Washing Event

The parameters needed to calculate the concentration in the water (C_w) in CFU/L for the face washing event include: the flow rate of the faucet (*FR*) in L/minute, the time for the face washing event (*T*) in minutes, the portion of water applied to the face (P_w), the surface area of the face (F_{SA}) and ocular region (O_{SA}) in cm^2, and the portion of eye left exposed during the event (P_E). Thus, the concentration in the water for the face washing event was calculated by transforming Equation 8 into Equation 9.

$$E_D = \frac{FR \times T \times P_W}{F_{SA}} \times O_{SA} \times P_E \times C_W \quad \text{Eq. 8}$$

$$C_W = \frac{E_D \times F_{SA}}{FR \times T \times P_W \times O_{SA} \times P_O} \quad \text{Eq. 9}$$

Hand Washing to Eye Touch Event

For the hand washing scenario, it was assumed that linear models developed to calculate the concentration of MS-2 transferred from liquid to skin were applicable to model the transfer of *P. aeruginosa* (Pitol, Bischel, Kohn, & Julian, 2017). Although *P. aeruginosa* is a gram-negative bacterium and MS-2 is traditionally used as a surrogate for enteric viruses, a previous study of the transfer efficiency of bacteria and viruses from porous and non-porous surfaces saws no substantial difference in transfer efficiencies between gram-negative bacteria and phages, suggesting that this published model study is suitable for application in the study herein (Lopez et al., 2013). The linear models are shown in Equations 10 and 11 where C_W is the concentration in the water in pathogens per milliliter, and *m* and *b* are the slope and intercept values calculated by Pitol et al. (2017). In the Pitol et al. study (2017) different slopes and intercepts were determined based on whether the virus was adsorbed or unadsorbed to the skin. This study assumed that if the hand washer dried their hands, the adsorbed pathogens would remain and be transferred to the eye, and if they did not dry, the unadsorbed pathogens would be transferred. In the drying scenarios, C_H (CFU/cm^2) was calculated with Equation 10. In the scenarios without drying, C_H was calculated with Equation 11. C_H was then multiplied by the surface area of a fingertip in cm^2 (FT), the number of transfers per day (T_F) and the transfer efficiency from fingertip to eye (T_E), as shown in Equation 12.

$$C_H = 10^{b1} {C_W}^{m1} \quad \text{Eq. 10}$$

$$C_H = 10^{b2} {C_W}^{m2} \quad \text{Eq. 11}$$

$$E_D = C_H \times FT \times T_F \times T_E \quad \text{Eq. 12}$$

For the reverse QMRA where the hand washer does dry their hands, Equations 10 and 12 were rearranged to Equation 13. Equations 11 and 12 were rearranged to Equation 14 to

represent the scenario when the hand washer does not dry their hands. The exposure assessment also accounted for any reduction in the pathogen that occurred from using bland or antimicrobial soap (*RD*) in log CFU. The final concentration was multiplied by a factor of 1000 to convert from CFU/mL to CFU/L.

$$C_W = 10^{\left[\frac{\left(\log\left(\frac{E_D}{FT \times T_F \times T_E}\right) + \left(\frac{RD}{FT}\right)\right) - b1}{m1}\right]} \times 1000 \quad \text{Eq. 13}$$

$$C_W = 10^{\left[\frac{\left[\log\left(\frac{E_D}{FT \times T_F \times T_E}\right) + \left(\frac{RD}{FT}\right)\right] - b2}{m2}\right]} \times 1000 \quad \text{Eq. 14}$$

Computation

To parameterize the inhalation exposure model a systematic literature review was conducted using the databases Web of Science, PubMed, and Google Scholar with keywords such as inhalation, *P. aeruginosa*, risk assessment, exposure dose, showering event, respiratory infection, tap water, opportunistic pathogen, respirable aerosols, and deposition. The same databases were used with the face washing and hand washing exposures but with keywords such as corneal exposure, *P. aeruginosa*, risk assessment, eye infection, keratitis, face washing event, time spent face washing, tap water, faucet flow rate, face surface area, ocular surface area, hand washing, transfer efficiency, contact lens, and washing efficiency. To the knowledge of these researchers, a risk assessment on a face washing event has not previously been completed for any pathogen. Thus, it was necessary to make several educated assumptions based on the data available.

In addition to the peer-reviewed resources found in the literature, the Exposure Factors Handbook (2011), and the Residential End Use Study (2016) were also used to create distributions for some of the aforementioned parameters. Sampling results from a newly renovated, low energy and low water use, residential home were also used to validate the

parameter distributions, as water use has declined in the residential sector. The green building has automated flowmeters installed allowing for the water usage pattern to be examined based on volume of water used, fixture events, duration, and flow rates. The water usage patterns in this home were used to corroborate the parameters determined for flow and duration of usage events (i.e. showering, face washing, etc.) in this study (Salehi et al., 2018). After parameters were determined, Oracle ® Crystal Ball was used to create distributions for the input parameters to account for variability and uncertainty. The dose response models and the parameters and distributions listed in Tables 6.2, 6.3, and 6.4 were used to forecast the concentrations of *P. aeruginosa* in the water resulting in the targeted risk thresholds for each scenario. Crystal Ball was run with a Monte Carlo sampling method, 10,000 trials and a seed of 999. A sensitivity analysis was completed for each scenario, with the Spearman rank correlation coefficient used to identify the input model parameters with the greatest contribution of variability and uncertainty in the calculated dependent variable - the final concentrations in the bulk water.

RESULTS

Exposure Dose

For the showering exposure, the average daily exposure dose was determined to be 302,750 CFU for an annual risk of infection equal to 1 infection in 10,000 persons. For the corneal exposure route for both the face washing and hand washing analyses, the average daily exposure dose was calculated to be 7.14E-04 CFU. Table 6.1 shows the annual risk of infection, daily risk of infection and exposure dose for all three scenarios.

Table 6.1: Exposure Doses Associated with an Annual Risk Level of 10^{-4}

Exposure Route	Annual Risk of Infection	Daily Risk of Infection	Daily Risk of Death*	Exposure Dose (CFU)
Showering	1.00E-04	2.74E-07	9.04E-08	302,750
Face Washing	1.00E-04	2.74E-07	n/a	7.14E-04
Hand Washing	1.00E-04	2.74E-07	n/a	7.14E-04

*Daily risk of death only calculated for inhalation route of exposure because only the inhalation dose response model had death as an endpoint of response

Exposure Assessment

Showering Event

Chattopadhyay et al. (2017) conducted a study using a showering apparatus to determine the partitioning coefficients (*PC*) for *Brevundimonas diminuta* and *P. aeruginosa* for varying water temperatures. For this study, the *PC* chosen was from the trials conducted at 37°C because this most closely approximates the average warm shower water temperature. The study used tap water spiked with two different initial concentrations of pathogens in the water (10^9 and 10^{10} CFU) resulting in two different partitioning coefficients. In the analysis herein, one value was set as a minimum and the other as a maximum in a uniform distribution. The same study also calculated the quantity of pathogens that were of a respirable size in these showering events (Chattopadhyay, Perkins, Shaw, & Nichols, 2017). This value ranged from 96.3 to 99.7% for the *P. aeruginosa* experiments, which was used to develop a uniform distribution to describe variability and uncertainty in F_{RA}.

The inhalation rate, *IR*, was represented with a triangular distribution using values from the Exposure Factors Handbook (2011). The likeliest value was the mean inhalation rate for adults over the age of 21 performing an activity with a light intensity and the maximum was the 95^{th} percentile value for this same group. The minimum value of 0.0042 m^3/minute was the mean inhalation rate for adults with a sedentary/passive activity level, to account for the population

that shower without any increase in inhalation rate (US Environmental Protection Agency, 2011). It should be noted that although based on adults, the average inhalation rate for a child also falls within this range. When considering shower duration, a uniform distribution was applied ranging from 7.8 minutes reported by the Residential End Use Study (2016) to 17 minutes reported by the Exposure Factors Handbook (2011). Finally, for the quantity deposited in the alveolar region (*RR*), the minimum and maximum values reported in a study analyzing lung deposition fractions in the alveolar regions for particles sized 1, 3, and 5 µm were used as a uniform distribution and are shown in Table 6.1 (U.S. EPA 2004).

Using the inhalation dose response model and the exposure equations and parameters shown in Table 6.2, a median concentration in the bulk water was estimated to be 6.07×10^{11} CFU/L. The mean, median and 95% confidence interval are listed in Table 6.5. A histogram of the distribution of plausible log concentrations determined for this scenario is shown in Figure 6.1.

Table 6.2: Showering Exposure Parameters and Distributions

Parameter	Average	Distribution	Source
Mortality Rate	0.33	Point Estimate	Sadikot et al. 2005; Hatchette et al. 2000
Dose Response, ***k*** parameter	4.12E-06	Point Estimate	Dean et al., 2019
Dose Response, k_{min} parameter	11	Point Estimate	Dean et al., 2019
Partitioning Coeffeicient, ***PC*** (L/m^3)	1.07E-05	Uniform: Minimum=4.56E-06, Maximum=1.69E-06	Chattopadhyay et al. 2017
Inhalation Rate, ***IR*** (m^3/minute)	0.013	Triangular: Minimum=0.0042, Likeliest=0.013, Maximum=0.017	Hines et al. 2014; U.S. EPA 2011
Respirable Fraction of Aerosols, F_{RA}	0.98	Uniform: Minimum=0.963, Maximum=0.997	Chattopadhyay et al. 2017
Deposition in Alveolar Region, ***RR***	0.37	Uniform: Minimum=0.32, Maximum=0.42	U.S. EPA 2004
Shower Time, ***T*** (minutes)	12.4	Uniform: Minimum=7.8, Maximum=17	U.S. EPA 2011 ; Hines et al. 2014

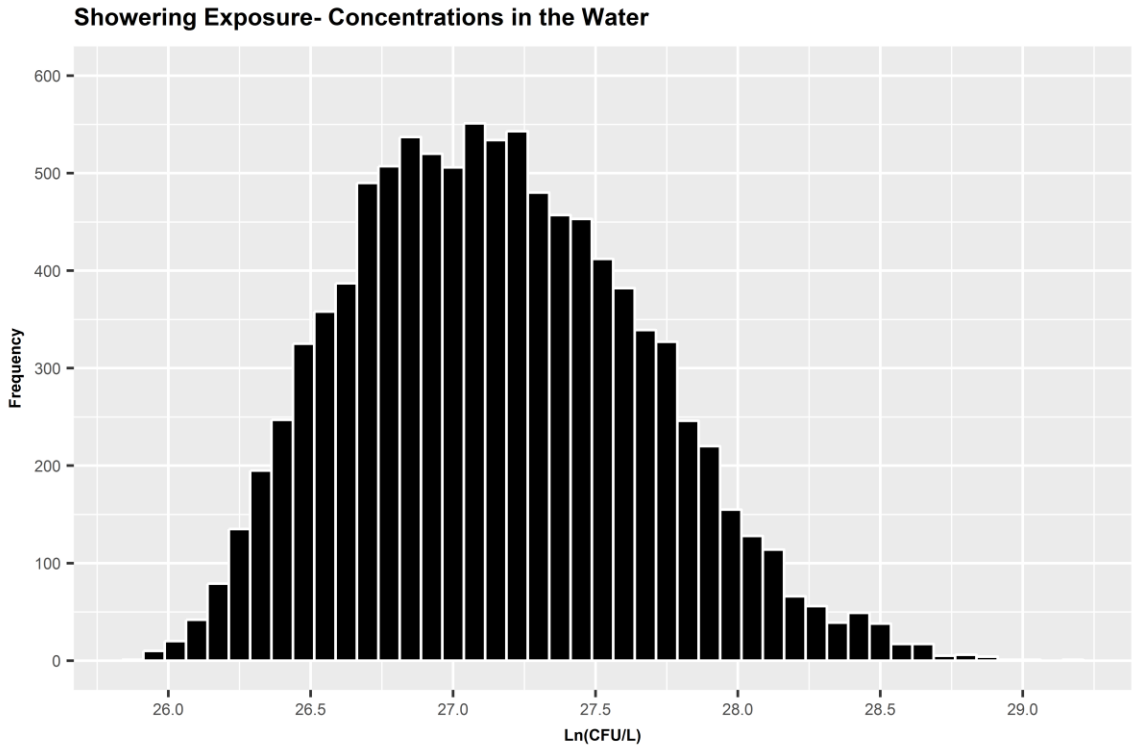


Figure 6.1: Natural log concentrations of *P. aeruginosa* in the bulk water resulting in a risk of infection from a showering exposure greater than the EPA mandated acceptable level generated from 10,000 iterations of the model

Face Washing Event

Once the mean exposure dose was calculated to be 7.18E-04 CFU, the concentration of *P. aeruginosa* was calculated with Equation 9. The face washing event time was based off of a study assessing water end uses in the United Arab Emirates that reported an average face washing time of 0.87 minutes (Chowdhury, El-Shorbagy, Ghanma, & El-Ashkar, 2015). To account for the uncertainty associated with this value, a triangular distribution was used with 0.87 minutes as the most likely value and an assumed minimum and maximum of 15 seconds and 1 minute, respectively. The faucet flow rate was also reported in the study as an average of 4 liters/minute. A study of fixture use and water quality in a residential green building observed average faucet flow rates from 2.1 to 6.3 liters/minute (Salehi et al., 2018). This range was used

as a uniform distribution for faucet flow rates as it encompasses the 4 liters/minute reported in the United Arab Emirates water end use study, as well as the average flow rate of 0.9 gallons/ minute or about 3.4 liters/minute reported in a study of high-efficiency new homes (W.B. Deoreo et al., 2011; William B Deoreo, Mayer, Dziegielewski, & Kiefer, 2016).

With the faucet flow rate and the length of the face washing event, the total volume of water used was calculated. However, a large portion of the water used while face washing is wasted, and not directly applied to the face. This quantity of unused water was estimated based on a study of ablution and different faucet types. With a tap with mechanical knobs, 47% of the tap water was wasted during ablution from the tap (Zaied, 2017). The facial surface area was estimated to be between 300 and 450 cm^2 (Yoon & Lee, 2016). Of that total surface area, the ocular region was assumed to be on average 1-3 cm^2 (Sotoyama, Villanueva, Jonai, & Saito, 1995). Finally, it was assumed that during a face washing event, a person's eye was open 10%, leading to the introduction of water into the eye. These values were considered typical. Any larger quantity of exposed eye (i.e. an eye wash event) would result in a greater risk of infection and a lower critical concentration of *P. aeruginosa* in the water causing that risk.

It was determined that a risk of 1 infection in 10,000 persons corresponds to a median concentration of 0.93 CFU/L of *P. aeruginosa* in the tap water. The mean, median, and 95% confidence interval for the exposure dose and pathogen concentration are listed in Table 6.5. Figure 6.3 represents the histogram of log concentration values in the water that result 1 infection in 10,000 persons annually.

Table 6.3: Face Washing Exposure Parameters and Distributions

Parameter	Value	Distribution	Source
Dose Response, ***α***	0.19	Point Estimate	Tamrakar, 2013
Dose Response, ***N50***	18500	Point Estimate	Tamrakar, 2013
Face Wash Duration, ***T*** (minutes)	0.87	Triangular: Minimum=0.25, Likeliest=0.87, Maximum= 1	Chowdhury et al., 2015; Assumption
Faucet Flow Rate (liters/minute), ***FR***	4.2	Uniform: Minimum=2.1, Maximum=6.3	Salehi et al., 2018
Portion of Water Applied to Face, ***PW***	0.53	Point Estimate	Zaied et al., 2017
Face Surface Area (cm^2), ***FA***	375	Uniform: Minimum=300, Maximum=450	Yoon & Lee, 2016
Ocular Surface Area (cm^2), ***OA***	2.0	Uniform: Minimum=1.0, Maximum=3.0	Sotoyama et al., 1995
Exposed Portion of Eye, ***PE***	0.125	Uniform: Minimum=0.0, Maximum=0.25	Assumption

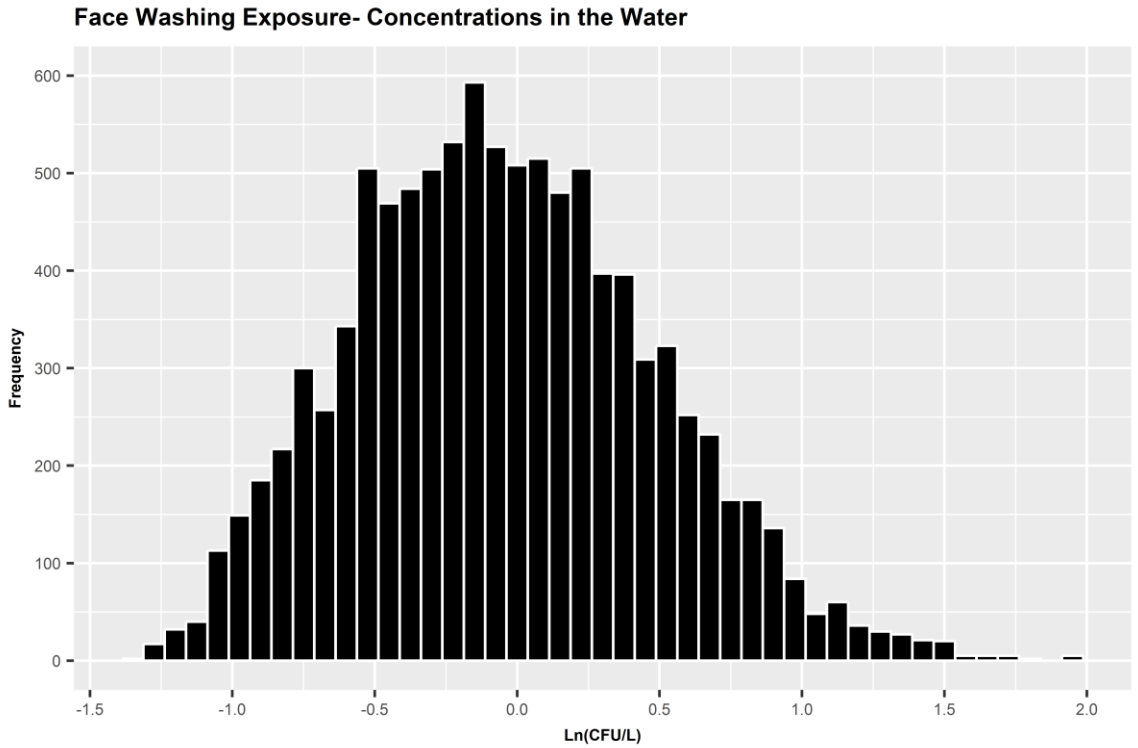


Figure 6.2: Natural log concentrations of *P. aeruginosa* in the bulk water resulting in a risk of infection from a face washing exposure greater than the EPA mandated acceptable level generated from 10,000 iterations of the model

Hand Washing to Eye Touch Event

The concentration on the hand after exposure to contaminated water was modeled using the results from a study on virus transfer at the liquid-skin interface. In this study, the unadsorbed fraction of viruses were the main driver of virus transfer. When the residual liquid was removed from the skin (i.e. proper hand drying occurred) only the adsorbed fraction remained. To model the effect of drying the hands before touching the eye, the adsorbed linear model was used to find concentration of pathogens on the hand (C_H) when the hands were dried (m_1 and b_1), and the unabsorbed model was used to find C_H when they were not dried (m_2 and b_2).

This scenario also took into account the removal of the pathogen based on the use of soap. A study that analyzed the log reduction of *E. coli* when bland or antimicrobial soap was used in a hand washing event was evaluated to represent potential *P. aeruginosa* removal (Jensen et al., 2017). The range of CFU log reductions for both kinds of soap are shown in Table 3 with triangular distributions. Fingertip surface area (*FT*) and transfer efficiency from fingertip to eye (T_E) were found from a study analyzing influenza infection risk from four exposure pathways, including contaminated hands touching facial membranes (Nicas & Jones, 2009). Nica and Jones (2009) assumed that the transfer efficiency from fingertip to lips of 35% was the same for fingertip to eye (Nicas & Jones, 2009; Rusin, Maxwell, & Gerba, 2002). This study makes the same assumption. The number of transfers in a day was assumed to be two, as the act of hand washing and immediately touching the eye after is likely most common for contact lens wearers and it is expected the contacts are inserted and removed each day.

Based on the assumptions and behaviors considered above, concentrations of concern in the water were calculated for six separate scenarios involving handwashing. These concentrations and scenarios are shown in Table 6.5. For the no soap and no drying scenario, a

median concentration of 137 CFU/L could result in a risk of infection of 1 in 10,000. If the handwasher used bland soap and dried afterwards, the median concentration in the water could be as high as 33,300 CFU/L before the risk threshold was met. As a highly conservative, health protective level of concern may be desirable in the absence of consumer behavior, the results from the no soap and no drying scenario were selected for comparison and reported in Table 6.6. Figure 6.3 represents the histogram of log concentration values for the no soap/no drying scenario.

Table 6.4: Hand Washing Exposure Parameters and Distributions

Parameter	Value	Distribution	Source
Dose Response, α	0.19	Point Estimate	Tamrakar, 2013
Dose Response, ***N50***	18500	Point Estimate	Tamrakar, 2013
Transfer Efficiency from Fingertip to Eye, T_E (%)	35	Point Estimate	Nicas and Jones 2009; Rusin et al. 2002
Fingertip Surface Area, *FT* (cm^2)	2	Point Estimate	Nicas and Jones 2009
Number of Transfers per Day, T_F	2	Point Estimate	Assumption
Bland Soap Log CFU Reduction, *RD*	2.22	Triangular: Minimum=1.91, 50%=2.22, Maximum=2.54	Jensen et al. 2017
Antimicrobial Soap Log CFU Reduction, *RD*	1.94	Triangular: Minimum=1.83, 50%=1.94, Maximum=2.10	Jensen et al. 2018
Adsorbed Fraction of Pathogen Slope, *m1*	1.1	Triangular: 2.5%=1.02, 50%=1.10, 97.5%=1.17	Pitol et al. 2017
Adsorbed Fraction of Pathogen Intercept, *b1*	-3.86	Triangular: 2.5%=-4.38 50%=-3.86, 97.5%=-3.33	Pitol et al. 2017
Unadsorbed Fraction of Pathogen Slope, *m2*	1.05	Triangular: 2.5%=0.99 50%=1.05, 97.5%=1.11	Pitol et al. 2017
Unadsorbed Fraction of Pathogen Intercept, *b2*	-2.33	Triangular: 2.5%=-1.97, 50%=-2.33, 97.5%=-1.13	Pitol et al. 2017

Table 6.5: Hand Washing Exposure Scenarios and Final Concentrations

Scenario		Concentration in Water (CFU/L)			
Soap Type	Drying	2.50%	Mean	Median	97.50%
No	No	4.43E+01	1.72E+02	1.37E+02	4.89E+02
Bland	No	4.95E+02	2.00E+03	1.58E+03	5.84E+03
Antimicrobial	No	3.85E+02	1.48E+03	1.17E+03	4.23E+03
No	Yes	1.08E+03	3.85E+03	3.23E+03	9.87E+03
Bland	Yes	1.07E+04	4.05E+04	3.33E+04	1.09E+05
Antimicrobial	Yes	8.32E+03	3.04E+04	2.52E+04	8.05E+04

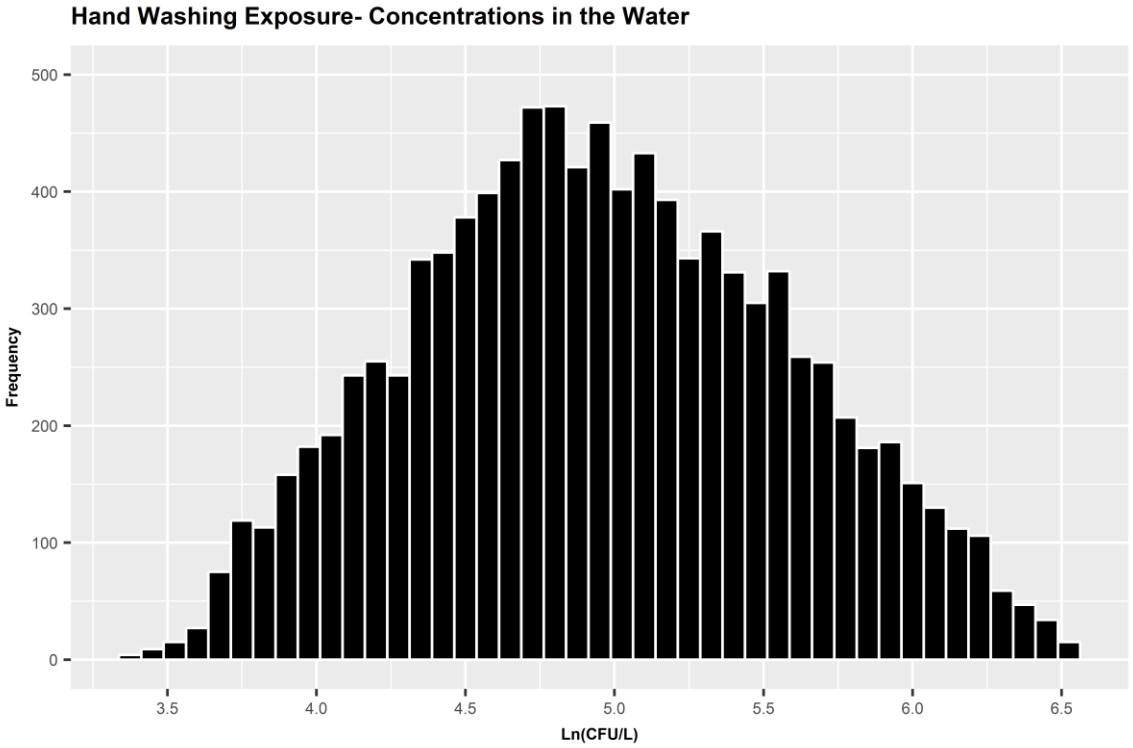


Figure 6.3: Natural log concentrations of *P. aeruginosa* in the bulk water that result in a risk of infection from a hand washing exposure (no soap or drying) greater than the EPA mandated acceptable level generated from 10,000 iterations of the model

Table 6.6: Summary Results of Exposure Assessments for all Three Scenarios

Exposure Route	Exposure Dose (CFU)	Concentration in the Bulk Water (CFU/L)			
		2.50%	Mean	Median	97.50%
Showering-Inhalation	302,750	2.55E+11	7.13E+11	6.04E+11	1.81E+12
Face Washing-Corneal	7.14E+04	3.73E-01	1.10E+00	0.92E+00	2.78E+00
Hand Washing-Corneal	7.14E+04	4.46E+01	1.72E+02	1.37E+02	4.92E+02

Sensitivity Analysis

A sensitivity analysis was completed for each exposure scenario to determine the uncertainty associated with each parameter in the reverse QMRA using the 10,000 Monte Carlo iterations. Spearman rank correlation coefficients were used to identify which parameters had the most influence on calculated threshold concentrations in the bulk water. The results of the sensitivity analysis for the showering exposure are shown in Figure 6.4. The partitioning coefficient had the highest Spearman rank correlation coefficient with a value of -0.71. The shower time and inhalation rate were also important predictive factors for risk, with correlation coefficients of -0.44 and -0.49.

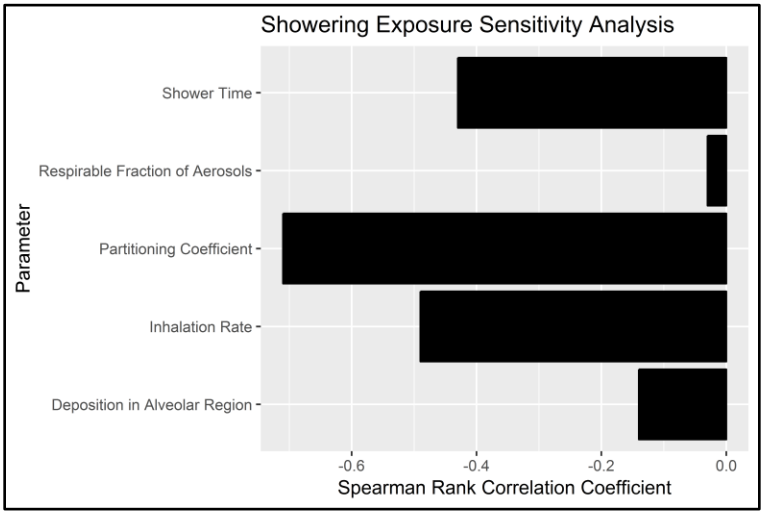


Figure 6.4: Sensitivity analysis of the showering exposure route

For the face washing scenario, the sensitivity analysis identified the faucet flow rate as the most sensitive factor for estimating the pathogen concentration in the water using the reverse QMRA model, with a correlation coefficient of -0.59. The ocular surface area and face wash time were the next most influential parameters with values of -0.58 and -0.47. This is shown in Figure 6.5.

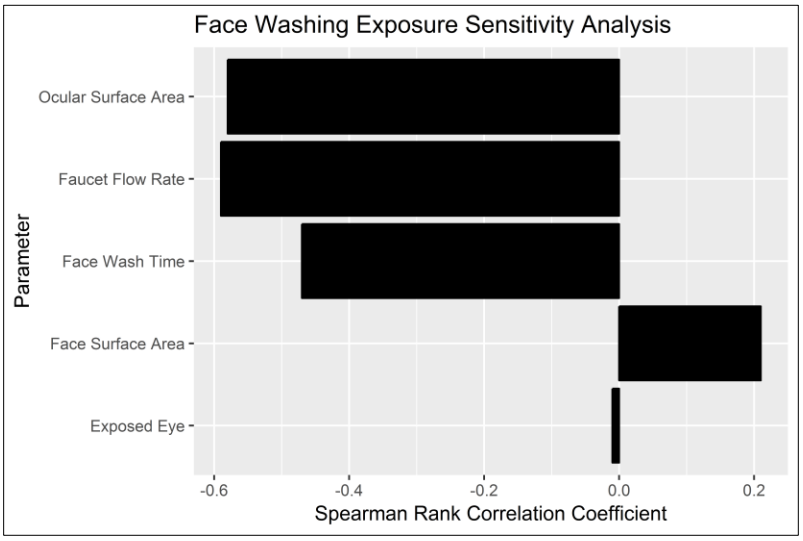


Figure 6.5: Sensitivity analysis of the face washing exposure route

Finally, the hand washing exposure only had distributions applied to the parameters involved in calculating the quantity of water adsorbed and unadsorbed on the hands, and the log reductions from soap use. For all scenarios, the intercept value (b_1 or b_2) used to calculate the concentration of pathogens transferred from the water to the hand in Equations 10 and 11 had the greatest influence on the concentration in the water as shown in Figure 6.6. For the main scenario of concern, no soap used and hands undried, the unadsorbed intercept had a correlation coefficient of -1.00. The uncertainty in the threshold concentration in the model is completely dependent on the uncertainty associated with the quantity of pathogen in residual water on the hands as expected. As the quantity of pathogens transferred from the water to the hand increases,

the concentration in the water responsible for an annual risk of 1 infection in 10,000 persons decreases.

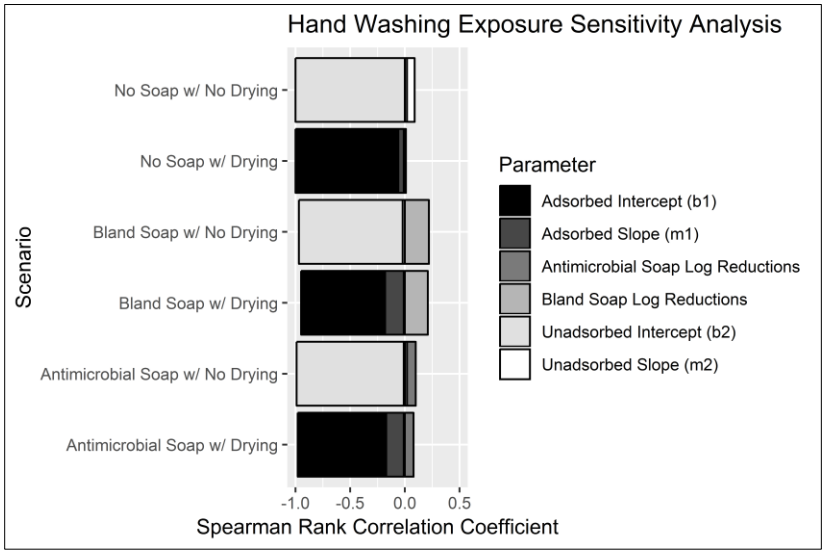


Figure 6.6: Sensitivity analysis of the hand washing exposure route

DISCUSSION

The results of this study indicate that for a showering exposure, a median concentration of 6.04×10^{11} CFU/L would result in 1 infection in 10,000 persons per year. However, for the face washing and hand washing exposures, median concentrations of 0.92 and 137 CFU/L, respectively, could result in 1 eye infection in 10,000 persons. The concentration level that needs to be monitored thus highly depends on the exposure route of concern. In a showering event, there are losses associated with each step across the exposure pathway (i.e. aerosolization, inhalation, deposition) and this allows for a much higher concentration in the water that may not result in significant risk. In addition, the dose response model for the inhalation route of exposure to *P. aeruginosa*, is a multi-hit model that estimates de minimis risks at lower concentrations and has a very high LD_{50} of 2,588,047 CFU (Dean, 2019). The face washing and hand washing exposures involve bacterium being directly applied to the eye, and this requires a much lower concentration in the water to result in the same level of risk. The beta-Poisson dose

response model for the corneal route of infection when compared to the multi-hit model estimates greater risk at lower concentrations and has an N_{50} of 18,500 CFU (Tamrakar, 2013).

This study provides threshold concentrations of concern for risk managers to use in sampling and monitoring protocols. Given that the pathogen proliferates in biofilms and sloughing events can lead to high concentrations of pathogens being suddenly present in the bulk water, biofilm growth should be controlled for. Neonatal units in Northern Ireland assessed the presence of *P. aeruginosa* in different tap assemblies and detected *P. aeruginosa* in 14% of the components, some that were colonized with up to $2.2x10^7$ CFU. For the face washing and hand washing events, a much lower concentration in the water can result in 1 infection in 10,000 persons. Such concentrations are more likely to occur based on published monitoring studies but may not be consistently prevalent. Groundwater surveys in Kansas, Oregon, Virginia, and Washington detected *P. aeruginosa* in 22 groundwater sources in densities ranging from 1 to 2,300 organisms per 100 ml (Allen & Geldreich, 1975; Mena & Gerba, 2009). A study of tap water in Greece detected *P. aeruginosa* in 9% of samples at a mean concentration of 7 CFU/100 mL(Mena & Gerba, 2009; Papapetropoulou, Iliopoulou, Rodopoulou, Detorakis, & Paniara, 1994). Based on this analysis, it is important for water managers to ensure that <1 CFU/L of *P. aeruginosa* is present at the tap. Additional monitoring studies are needed within premise plumbing systems to build confidence in the maintenance of the biological stability of the drinking water post-treatment. Effective management of *P. aeruginosa* in drinking water requires a better understanding both the baseline concentrations in the bulk water and potential intermittent high concentrations associated with biofilm detachment.

This was the first inhalation risk assessment for *P. aeruginosa*, which expands the understanding of the most significant exposure pathway and risks for immunocompromised

populations. Previous risks assessments for *P. aeruginosa* were limited by a lack of applicable dose-response data. As with all risk assessment, there are several limitations that should be addressed. The dose response model developed for the inhalation exposure was based on death as an endpoint of response, requiring mortality and morbidity rates to estimate the risk of infection (Dean, 2019). While death is the most stable endpoint for modeling dose response there is variability and uncertainty associated with the morbidity and mortality rates across the population. The showering event was analyzed based on a healthy individual's response to the doses of *P. aeruginosa* in the water. For immunocompromised individuals, the population primarily affected by the pathogen, it is expected that a much lower concentration in the water would result in 1 infection in 10,000 persons per year. Potential approaches to address this irreducible uncertainty could be to apply the 95^{th} percentile of the parameter estimates to the dose response model and to use the recorded mortality or morbidity rates for the immunocompromised. However, these methods may not be applicable in this situation, as the dose response curve for the immunocompromised population exposed to *P. aeruginosa* via the inhalation route is expected to be shaped differently than the curve for the immunocompetent. Excluding the dose response parameters, the sensitivity analysis identified the main variables affecting the result of the exposure assessment for the showering scenario- the partitioning coefficient, the inhalation rate, and the time spent showering. The partitioning coefficient had the greatest influence and this was expected considering it is assumed to be highly variable with type of showerhead, flow rate, water quality, contaminant characteristics, etc. (Chattopadhyay et al., 2017). It is therefore difficult to further refine the uncertainty in estimates of *PC* without specific future experimentation for different scenarios based on the influential factors described (Chattopadhyay et al., 2017). The inhalation rate and time spent showering are inherently

variable values across the population and additional information is unlikely to reduce model uncertainty associated with these values.

Several assumptions were made in the face washing risk assessment in the absence of previous existing work. The results of the assessment indicated that a person washing their face with a 10% exposed ocular surface area on average could have a risk of infection of 10^{-4} per year when the concentration in the water is as low as 0.92 CFU/L. This risk would logically increase if a greater portion of the eye was left open and decrease the more the eye was kept closed. This dependence is reflected in the sensitivity analysis as the ocular surface area is the parameter with the second greatest effect on the concentration in the water. The smaller the eye, the lower the exposed ocular surface area and the lower quantity of water entering the eye during the face washing event to initiate infection. The quantity of water wasted and not applied to the face during a face washing event was estimated in this analysis based on an ablution study, because ablution from taps is a repeated daily activity that includes washing the face. However ablution also includes washing other parts of the body and thus this was only estimated to be a similar representation of water loss in face washing. Other variables with a strong influence on the risk assessment were the time spent face washing and the faucet flow rate. These two parameters were used to calculate the expected portion of water the eye is exposed to and as such, if these variables decrease, so does the risk of infection. The faucet flow rate is variable across the population but based on fixture type. The time spent face washing is inherently variable across the population and though the estimate could be refined with additional studies, the contribution of uncertainty in the risk assessment is unlikely to change.

Finally, an exposure scenario where an individual that washed their hands and immediately touched their eye was assessed. Not only is this action common for contact lens

wearers but, an observational study of students performing office work saw the average individual touch their eyes about 2.5 times per hour (Nicas & Best, 2008; Nicas & Jones, 2009). This assessment found that if a person washed their hands without soap and allowed residual water to remain on their hands before touching their eye, a concentration of 137 CFU/L in the water could result in a risk of infection of 10^{-4}. However, if the faucet user had washed with bland soap and dried their hands afterwards, a concentration of 33,300 CFU/L would be needed to have the same level of risk. A person not completely drying their hands is a plausible scenario as observed in a study of bacterial transfer after hand washing where the drying habits of male and females in washrooms were recorded. Male washroom users dried their hands for an average of 3.5 seconds with cloth towels and 17 seconds under hot air dryers compared to female users that spent on average 5.2 and 13.3 seconds with the cloth and air dryers, respectively. The study determined that 5 seconds with cloth towels would achieve only 85% dryness and 20 seconds using an air dryer would achieve only 70% dryness (C. Huang, Ma, & Stack, 2012; Patrick, Findon, & Miller, 1997). A sensitivity analysis identified the Log_{10} transformed slope and intercept parameters (*m* and *b*) from the linear regression models used to describe virus transfer from liquid to skin as having the greatest influence on risk of infection. Depending on the scenario, the linear model intercept had a correlation coefficient between -0.85 and -1.00. For the no soap and no drying scenario, the uncertainty in the concentration in the water was entirely dependent on the uncertainty of the intercept value.

A limitation of this analysis is that the model used to calculate the transfer of *P. aeruginosa* from water to hand is based on the transfer of the bacteriophage MS-2. A study of bacteria and virus transfer from surfaces saw no substantial difference in transfer efficiencies between gram-negative bacteria and phages from surfaces (Lopez et al., 2013). Another study of

surface-to-hand and fingertip-to-mouth transfer efficiency determined similar fingertip-to-mouth transfer efficiencies for gram-negative bacteria and phages of 33.97% and 33.90%, respectively, suggesting similar behavior (Rusin et al., 2002). For most of the surface-to-hand experiments in the same study, the phage was transferred more efficiently than the gram-negative bacterium, suggesting that its use as a surrogate in this analysis may yield conservative results (Rusin et al., 2002). Any future studies of bacterium transfer from water to skin should be incorporated into the model, as it has a strong influence on the model results. Additionally, the study used to model the transfer of *P. aeruginosa* from water to skin addressed the transfer that occurs in stagnant water (Pitol et al., 2017). Future studies of running water would better capture the transfer that occurs during a hand washing event. Finally, the log reductions from bland and antimicrobial soap removal was based on the removal of *E. coli* during a hand washing event. Like *P. aeruginosa*, *E. coli* is a rod-shaped, gram-negative bacterium and the nonpathogenic strain chosen by Jensen et al. (2018) was selected because it is a well-established surrogate for bacteria that may be transferred to the hands when handling raw foods. In the absence of data for the effects of soap use on *P. aeruginosa* reduction specifically, reductions based on *E. coli* were used as a surrogate in this analysis.

With further experiments and data collection the threshold concentrations proposed in this study can be further refined. The current results are based on the state of science in understanding the parameters impacting risks in these scenarios. The results provide critical information to individuals responsible for monitoring pathogen levels in drinking water, especially when a population known to be immunocompromised is in consideration for cases like nursing homes or hospitals.

CONCLUSION

The results of this study provide threshold concentrations of concern for *P. aeruginosa* in premise plumbing systems when considering showering, hand washing, and face washing exposure scenarios. *P. aeruginosa* is an opportunistic pathogen that can cause a variety of infections including pneumonia and bacterial keratitis. It is ubiquitous and known to thrive in the biofilms of premise plumbing systems, making monitoring protocols incredibly important for risk managers to appropriately protect human health. The lowest range of concentrations responsible for a risk of 1 infection in 10,000 persons was from the face washing scenario, with a 95% confidence interval of 0.37-2.78 CFU/L. This range should serve as the threshold of *P. aeruginosa* of concern in premise plumbing systems to inform the remediation and monitoring protocols for risk managers. Faucets and fixtures that facilitate the direct application of water to the face and eyes should be of particular concern. These results demonstrate that reverse QMRAs can play an important role in prioritizing pathogen monitoring and treatment within man-made water infrastructure.

CHAPTER 7: CONCLUSIONS

This thesis has developed dose response models for three opportunistic pathogens of concern. Unique to this work, is the development of dose response models for the exposure routes that most closely mimic possible exposures to *N. fowleri*, *Acanthamoeba* spp., and *P. aeruginosa* from drinking water use. The recommended dose response models in these analyses for the intranasal, inhalation, and corneal exposure routes are consistent with possible exposures from face washing, showering, bathing, and hand washing events.

In addition to providing the needed dose response models for the exposure routes of concern, this analysis also completed a reverse QMRA for *P. aeruginosa*. The reverse QMRA addressed face washing, hand washing, and showering exposure events, and calculated threshold concentrations in the bulk water that could be responsible for an annual risk of infection of 10^{-4}. The results indicated that if a very conservative estimate is required for monitoring, the risk of bacterial keratitis should be used as a threshold for *P. aeruginosa* in drinking water. However, it is important to address that all infections are not created equal. Although this assessment addresses the same risk threshold for eye infections and lung infections, it is possible that a lower allowable threshold would be preferred for pneumonia-related infections, as they have higher associated mortality rates and the long-term sequelae of infection may have more serious effects on day-to-day life.

P. aeruginosa is not currently a prioritized drinking water pathogen and this work indicates that greater attention may need to be paid to the bacterial opportunistic pathogen. The results from the reverse QMRA indicate that a concentration of 1 CFU/L at the tap may pose a significant risk to human health. A possible management solution may be to implement regulations at the treatment facility that require minimal levels of *P. aeruginosa* be able to enter

the distribution system. The feasibility of monitoring *P. aeruginosa* levels to ensure that a <1 CFU/L concentration is maintained is not high, however, because of the detection limits, time-required, and expenses involved in possible sampling protocols. Other risk management strategies and techniques need to be investigated to limit the presence of *P. aeruginosa* in distribution systems and premise plumbing.

CHAPTER 8: FUTURE WORK

Quantifying the risk posed by opportunistic pathogens for all possible exposure scenarios is a critical need. As demonstrated by the reverse QMRA for *P. aeruginosa*, the exposure route dramatically affects the concentrations of concern in the bulk water. The results of this analysis can be used by decision makers to influence management decisions for treatment protocols, monitoring plans, and premise plumbing design. Future work should address threshold concentrations of concern for the inhalation route of exposure to *P. aeruginosa* for the immunocompromised population. In particular, it is not believed that the dose response model developed in this study for the inhalation route of exposure to *P. aeruginosa* is currently applicable to the immunocompromised population. It is unlikely that the same threshold behavior would be observed if *P. aeruginosa* was introduced to a host without the same level of immune system defenses. Options for modifying the dose response model to account for this more vulnerable subset is a necessary next step.

This work should also be expanded upon to translate the bulk water threshold concentrations developed in this study into biofilm concentrations, as the pathogens discussed in this thesis are known to reside within the biofilm. Although some of the threshold concentrations calculated in this study may not be common in bulk water samples, it is possible that much greater concentrations are present within biofilms and sloughing events could result in drastic changes in the concentrations of pathogens in the bulk water in a short window of time. The causes of sloughing events are not always well understood, which poses a challenge for monitoring. Thus further research is needed to understand how to limit the presence of biofilms and pathogen growth, to prevent the possible occurrence of concentrations of pathogens that threaten human health. An element of this research should include the investigation of

opportunistic pathogen persistence in these biofilm and bulk water environments, as this will affect the development of effective risk management strategies. The threshold concentrations in this work and the future risk assessments that use the developed dose response models can be used to inform policy decisions and help ensure that safe drinking water is provided to the public.

REFERENCES

REFERENCES

Allen, M. J., & Geldreich, E. E. (1975). Bacteriological Criteria for Ground-Water Quality. *Ground Water*, *13*(1), 45–52.

Badenoch, P. R., Johnson, A. M., Christy, P. E., & Coster, D. J. (1990). Pathogenicity of Acanthamoeba and a Corynebacterium in the Rat Cornea. *Archives of Ophthalmology*, *108*(1), 107–112. https://doi.org/10.1001/archopht.1990.01070030113040

Badenoch, P. R., Johnson, A. M., Christy, P. E., & Coster, D. J. (1991). A Model of Acanthamoeba keratitis in the Rat. *Review of Infectious Diseases*, *13*(Suppl. 5), S445.

Barbeau, J., & Buhler, T. (2001). Biofilms augment the number of free-living amoebae in dental unit waterlines. *Research in Microbiology*, *152*(8), 753–760. https://doi.org/10.1016/S0923-2508(01)01256-6

Bartrand, T. A., Causey, J. J., & Clancy, J. L. (2014). Naegleria fowleri: An emerging drinking water pathogen. *Journal - American Water Works Association*, *106*(10), E418–E432. https://doi.org/10.5942/jawwa.2014.106.0140

Bédard, E., Fey, S., Charron, D., Lalancette, C., Cantin, P., Dolcé, P., … Prévost, M. (2015). Temperature diagnostic to identify high risk areas and optimize Legionella pneumophila surveillance in hot water distribution systems. *Water Research*, *71*, 244–256. https://doi.org/10.1016/j.watres.2015.01.006

Bédard, E., Prévost, M., & Déziel, E. (2016). Pseudomonas aeruginosa in premise plumbing of large buildings. *MicrobiologyOpen*, *5*(6), 937–956. https://doi.org/10.1002/mbo3.391

Benedict, K. M., Reses, H., Vigar, M., Roth, D. M., Roberts, V. A., Mattioli, M., … Hill, V. R. (2017). Surveillance for Waterborne Disease Outbreaks Associated with Drinking Water-United States, 2013-2014. *CDC Morbidity and Mortality Weekly Report*, *66*(44), 1216–1221.

Bichai, F., Payment, P., & Barbeau, B. (2008). Protection of waterborne pathogens by higher organisms in drinking water: a review. *Canadian Journal of Microbiology*, *54*(7), 509–524. https://doi.org/10.1139/W08-039

Billinger, M. E., Olivier, K. N., Viboud, C., Montes De Oca, R., Steiner, C., Holland, S. M., & Prevots, D. R. (2009). Nontuberculous mycobacteria-associated lung disease in hospitalized persons, United States, 1998-2005. *Emerging Infectious Diseases*, *15*(10), 1562–1569. https://doi.org/10.3201/eid1510.090196

Blair, B., Sarkar, P., Bright, K. R., Marciano-Cabral, F., & Gerba, C. P. (2008). Naegleria fowleri in Well Water. *Emerging Infectious Diseases*, *14*(9), 1499–1500.

https://doi.org/10.3201/eid1409.080143

Brazeau, R. H., & Edwards, M. A. (2013). Role of Hot Water System Design on Factors Influential to Pathogen Regrowth: Temperature, Chlorine Residual, Hydrogen Evolution, and Sediment. *Environmental Engineering Science*, *30*(10), 617–627. https://doi.org/10.1089/ees.2012.0514

Bright, K. R., & Gerba, C. P. (2017). Review: Occurrence of the pathogenic amoeba Naegleria fowleri in groundwater. *Hydrogeology Journal*, *25*(4), 953–958. https://doi.org/10.1007/s10040-017-1582-4

Bukh, A. S., & Roslev, P. (2014). Mycobacterium avium complex in day care hot water systems, and persistence of live cells and DNA in hot water pipes. *Current Microbiology*, *68*(4), 428–439. https://doi.org/10.1007/s00284-013-0493-4

Cerva, L. (1967a). Intracerebral Inoculation of Experimental Animals in Pathogenetical Studies of Hartmanella castellanii. *Foli Parasitologica (Praha)*, *14*, 171–175.

Cerva, L. (1967b). Intranasal, Intrapulmonary, and Intracardial Inoculation of Experimental Animals with Hartmanella castellanii. *Folia Parasitologica (Praha)*, *14*, 207–215.

Cervero-Aragó, S., Rodríguez-Martínez, S., Canals, O., Salvadó, H., & Araujo, R. M. (2013). Effect of thermal treatment on free-living amoeba inactivation. *Journal of Applied Microbiology*, *116*(3), 728–736. https://doi.org/10.1111/jam.12379

Cervero-Aragó, S., Rodríguez-Martínez, S., Puertas-Bennasar, A., & Araujo, R. M. (2015). Effect of common drinking water disinfectants, chlorine and heat, on free Legionella and amoebae-associated Legionella. *PLoS ONE*, *10*(8), 1–18. https://doi.org/10.1371/journal.pone.0134726

Chattopadhyay, S., Perkins, S. D., Shaw, M., & Nichols, T. L. (2017). Evaluation of exposure to Brevundimonas diminuta and Pseudomonas aeruginosa during showering. *Journal of Aerosol Science*, *114*(April), 77–93. https://doi.org/10.1016/j.jaerosci.2017.08.008

Chowdhury, R. K., El-Shorbagy, W., Ghanma, M., & El-Ashkar, A. (2015). Quantitative assessment of residential water end uses and greywater generation in the City of Al Ain. *Water Science and Technology: Water Supply*, *15*(1), 114–123. https://doi.org/10.2166/ws.2014.090

Cohen, T. S., & Prince, A. S. (2013). Activation of inflammasome signaling mediates pathology of acute P . aeruginosa pneumonia, *123*(4). https://doi.org/10.1172/JCI66142.1630

Coleman, M. E., Marks, H. M., Hertzberg, R. C., Michele, M., Coleman, M. E., Marks, H. M., & Hertzberg, R. C. (2017). Human and Ecological Risk Assessment : An International Mechanistic modeling of salmonellosis : Update and future directions. *Human and Ecological Risk Assessment*, *23*(8), 1830–1856.

https://doi.org/10.1080/10807039.2017.1356682

Coleman, M. E., Thran, B., Morse, S. S., Hugh-jones, M., & Massulik, S. (2008). Inhalation Anthrax: Dose Response and Risk Analysis, *6*(2), 147–160. https://doi.org/10.1089/bsp.2007.0066

Cope, J. R., Ratard, R. C., Hill, V. R., Sokol, T., Causey, J. J., Yoder, J. S., … Beach, M. J. (2015). The first association of a primary amebic meningoencephalitis death with culturable naegleria fowleri in tap water from a US treated public drinking water system. *Clinical Infectious Diseases*, *60*(8), e36–e42. https://doi.org/10.1093/cid/civ017

Cope, J., Ratard, R. C., Hill, V. R., Sokol, T., Causey, J. J., Yoder, J. S., … Beach, M. J. (2015). The First Association of a Primary Amebic Meningoencephalitis Death with Culturable Naegleria fowleri in Tap Water from a U.S. Treated Public Drinking Water Systems. *Clinical Infectious Diseases*, *60*(8), e36–e42.

Cornforth, D. M., Matthews, A., Brown, S. P., & Raymond, B. (2015). Errors in Pathogen Risk Assessment due to the Failure of the Independent Action Hypothesis, 1–13. https://doi.org/10.1371/journal.ppat.1004775

Culbertson, C. G., Ensminger, P. W., & Overton, W. M. (1965). The Isolation of Additional Strains of Pathogenic Hartmanella sp. (Acanthamoeba). *The American Journal of Clinical Pathology*, *43*(4), 383–387.

Culbertson, C. G., Ensminger, P. W., & Overton, W. M. (1966). Hartmannella (Acanthamoeba): Experimental Chronic, Granulomatous Brain Infections Produced by New Isolates of Low Virulence. *The American Journal of Clinical Pathology*, *46*(3), 305–314.

Culbertson, C. G., Holmes, D. H., & Overton, W. M. (1965). Hartmanella castellani (Acanthamoeba sp.). *The American Journal of Clinical Pathology*, *43*(4), 361–364.

Dai, D., Rhoads, W. J., Edwards, M. A., & Pruden, A. (2018). Shotgun Metagenomics Reveals Taxonomic and Functional Shifts in Hot water microbiome due to temperature setting and stagnation. *Frontiers in Microbiology*, *9*(NOV), 1–17. https://doi.org/10.3389/fmicb.2018.02695

Dean, K. J. (2019). *Modeling Risk for Intranasal, Inhalation, and Corneal Exposures to Opportunistic Pathogens of Concern in Drinking Water*. Michigan State University. Submitted.

Dean, K. J., & Mitchell, J. (2019). Reverse QMRA for Pseudomonas aeruginosa in premise plumbing to inform risk management. *Manuscript Submitted for Publication*.

Dean, K. J., Tamrakar, S., Huang, Y., Rose, J., & Mitchell, J. (2019). Modeling the dose-response relationship of Acanthamoeba spp. *Manuscript Submitted for Publication*.

Dean, K. J., Weir, M. H., & Mitchell, J. (2019). Development of a dose–response model for Naegleria fowleri. *Journal of Water and Health*, *17*(1), 63–71. https://doi.org/10.2166/wh.2018.181

Deoreo, W. B., Mayer, P. W., Duffield, M. K., Martien, L., Hayden, M., Davis, R., & Funk, A. (2011). *Analysis of Water Use in New Single Family Homes*. Aquacraft Inc. Water Engineering and Management. Boulder, CO.

Deoreo, W. B., Mayer, P. W., Dziegielewski, B., & Kiefer, J. C. (2016). *Residential End Uses of Water, Version 2*. *Water Research Foundation*. https://doi.org/4309b

Donohue, M. J., Mistry, J. H., Donohue, J. M., Oconnell, K., King, D., Byran, J., … Pfaller, S. (2015). Increased frequency of nontuberculous mycobacteria detection at potable water taps within the United States. *Environmental Science and Technology*, *49*(10), 6127–6133. https://doi.org/10.1021/acs.est.5b00496

Driscoll, J. A., Brody, S. L., & Kollef, M. H. (2007a). The epidemiology, pathogenesis and treatment of Pseudomonas aeruginosa infections. *Drugs*, *67*(3), 351–368. https://doi.org/10.2165/00003495-200767030-00003

Driscoll, J. A., Brody, S. L., & Kollef, M. H. (2007b). The Epidemiology, Pathogenesis and Treatment of Pseudomonas aeruginosa Infections. *Drugs*, *67*(3), 351–368.

Druett, H. A. (1952). Bacterial Invasion. *Nature*, *170*, 288.

Dupuy, M., Mazoua, S., Berne, F., Bodet, C., Garrec, N., Herbelin, P., … Héchard, Y. (2011). Efficiency of water disinfectants against Legionella pneumophila and Acanthamoeba. *Water Research*, *45*(3), 1087–1094. https://doi.org/10.1016/j.watres.2010.10.025

Environmental Protection Agency. (2003). National Primary Drinking Water Regulations. *Code of Federal Regulations*, *40*(141), 341–565. Retrieved from https://www.epa.gov/ground-water-and-drinking-water/national-primary-drinking-water-regulations

Falkinham Joseph, I. I. I. (2013). Ecology of Nontuberculous Mycobacteria—Where Do Human Infections Come from? *Seminars in Respiratory and Critical Care Medicine*, *34*(01), 95–102. Retrieved from http://www.thieme-connect.de/DOI/DOI?10.1055/s-0033-1333568%5Cnpapers2://publication/doi/10.1055/s-0033-1333568

Falkinham III, J. O. (2013). Reducing Human Exposure to Mycobacterium avium. *Annals of the American Thoracic Society*, *10*(4), 378–382. https://doi.org/10.1513/AnnalsATS.201301-013FR

Falkinham III, J. O., Hilborn, E. D., Arduino, M. J., Pruden, A., & Edwards, M. A. (2015). Review Epidemiology and Ecology of Opportunistic Premise Plumbing Pathogens : Legionella pneumophila, Mycobacterium avium, and Pseudomonas aeruginosa. *Environmental Health Perspectives*, *123*(8), 749–758.

Falkinham, J. O. (2015). Common Features of Opportunistic Premise Plumbing Pathogens. *International Journal of Environmental Research and Public Health*, *12*(5), 4533–4545. https://doi.org/10.3390/ijerph120504533

Falkinham, J. O., Norton, C. D., & Mark, W. (2001). Factors Influencing Numbers of Mycobacterium avium , Mycobacterium intracellulare , and Other Mycobacteria in Drinking Water Distribution Systems Factors Influencing Numbers of Mycobacterium avium , Mycobacterium intracellulare , and Other Mycobacteria in. *Applied and Environmental Microbiology*, *67*(3), 1225–1231. https://doi.org/10.1128/AEM.67.3.1225

Falkinham, J., Pruden, A., & Edwards, M. (2015). Opportunistic Premise Plumbing Pathogens: Increasingly Important Pathogens in Drinking Water. *Pathogens*, *4*(2), 373–386. https://doi.org/10.3390/pathogens4020373

Flemming, H., Wingender, J., & Szewzyk, U. (2011). *Biofilm Highlights*. *Springer Series on Biofilms*. Springer Berlin Heidelberg.

Gerba, C. P., Nwachuku, N., & Riley, K. R. (2003). Disinfection resistance of waterborne pathogens on the United States Environmental Protection Agency's Contaminant Candidate List (CCL). *Journal of Water Supply: Research and Technology - AQUA*, *52*(2), 81–94. https://doi.org/10.2166/aqua.2003.0009

Haas, C. N., Rose, J. B., & Gerba, C. P. (2014). *Quantitative Microbial Risk Assessment* (Second). J. Wiley and Sons.

Haas, C., Rose, J., & Gerba, C. (2014). *Quantitative Microbial Risk Assessment* (Second). Hoboken, New Jersey: John Wiley & Sons, Inc.

Hamilton, K. A., Ahmed, W., Toze, S., & Haas, C. N. (2017). Human health risks for Legionella and Mycobacterium avium complex (MAC) from potable and non-potable uses of roof-harvested rainwater. *Water Research*, *119*, 288–303. https://doi.org/10.1016/j.watres.2017.04.004

Hamilton, K. A., Hamilton, M. T., Johnson, W., Jjemba, P., Bukhari, Z., LeChevallier, M., & Haas, C. N. (2018). Health risks from exposure to Legionella in reclaimed water aerosols: Toilet flushing, spray irrigation, and cooling towers. *Water Research*, *134*, 261–279. https://doi.org/10.1016/j.watres.2017.12.022

Hamilton, K. A., Weir, M. H., & Haas, C. N. (2017). Dose response models and a quantitative microbial risk assessment framework for the Mycobacterium avium complex that account for recent developments in molecular biology, taxonomy, and epidemiology. *Water Research*. Elsevier Ltd. https://doi.org/10.1016/j.watres.2016.11.053

Hatchette, T. F., Gupta, R., & Marrie, T. J. (2000). *Pseudomonas aeruginosa* Community-Acquired Pneumonia in Previously Healthy Adults: Case Report and Review of the

Literature. *Clinical Infectious Diseases*, *31*(6), 1349–1356. https://doi.org/10.1086/317486

Hoffman, R., Marshall, M. M., Gibson, M. C., & Rochelle, P. A. (2009). Prioritizing pathogens for potential future regulation in drinking water. *Environmental Science and Technology*. https://doi.org/10.1021/es803532k

Hoffmann, R., & Michel, R. (2001). Distribution of free-living amoebae (FLA) during preparation and supply of drinking water. *International Journal of Hygiene and Environmental Health*, *203*(3), 215–219. https://doi.org/10.1078/S1438-4639(04)70031-0

Hraiech, S., Hiblot, J., Lafleur, J., Lepidi, H., Papazian, L., Rolain, J., … Chabriere, E. (2014). Inhaled Lactonase Reduces Pseudomonas aeruginosa Quorum Sensing and Mortality in Rat Pneumonia, *9*(10), 1–8. https://doi.org/10.1371/journal.pone.0107125

Huang, C., Ma, W., & Stack, S. (2012). The hygienic efficacy of different hand-drying methods: A review of the evidence. *Mayo Clinic Proceedings*, *87*(8), 791–798. https://doi.org/10.1016/j.mayocp.2012.02.019

Huang, Y. (2013). Naegleria fowleri: Dose Response Models. Retrieved March 13, 2019, from http://qmrawiki.canr.msu.edu/index.php/Naegleria_fowleri:_Dose_Response_Models

Irvin, C. G., & Bates, J. H. T. (2003). Measuring the lung function in the mouse: The challenge of size. *Respiratory Research*, *4*, 1–9. https://doi.org/10.1186/rr199

Janardan, K. G. (1986). A Lagrange-gamma multihit dose-response model for risk assessment. *Mathematical Biosciences*, *80*(2), 209–222. https://doi.org/10.1016/0025-5564(86)90045-3

Jensen, D. A., Macinga, D. R., Shumaker, D. J., Bellino, R., Arbogast, J. W., & Schaffner, D. W. (2017). Quantifying the Effects of Water Temperature, Soap Volume, Lather Time, and Antimicrobial Soap as Variables in the Removal of *Escherichia coli* ATCC 11229 from Hands. *Journal of Food Protection*, *80*(6), 1022–1031. https://doi.org/10.4315/0362-028X.JFP-16-370

John, D. T., & Hoppe, K. L. (2017). Susceptibility of Wild Mammals to Infection with Naegleria fowleri Author (s): David T . John and Kenneth L . Hoppe Published by : Allen Press on behalf of The American Society of Parasitologists Stable URL : http://www.jstor.org/stable/3282806 digitize, *76*(6), 865–868.

John, D. T., & Nussbaum, S. L. (1983). Naegleria fowleri Infection Acquired by Mice through Swimming in Amebae-Contaminated Water. *The Journal of Parasitology*, *69*(5), 871–874.

Jones, M. B., Jani, R., Ren, D., Wood, T. K., & Blaser, M. J. (2005). Inhibition of Bacillus anthracis Growth and Virulence-Gene Expression by Inhibitors of Quorum-Sensing, *10016*, 1881–1888.

Karrasch, S., Eder, G., Bolle, I., Tsuda, A., & Schulz, H. (2009). Breath-by-breath measurement

of particle deposition in the lung of spontaneously breathing rats. *Journal of Applied Physiology*, *107*(4), 1293–1299. https://doi.org/10.1152/japplphysiol.00096.2009

Kerr, K. G., & Snelling, A. M. (2009). Pseudomonas aeruginosa: a formidable and ever-present adversary. *Journal of Hospital Infection*, *73*(4), 338–344. https://doi.org/10.1016/j.jhin.2009.04.020

Kilvington, S., Gray, T., Dart, J., Morlet, N., Beeching, J. R., Frazer, D. G., & Matheson, M. (2004). Acanthamoeba Keratitis: The Role of Domestic Tap Water Contamination in the United Kingdom. *Investigative Ophthalmology and Visual Science*, *45*(1), 165–169. https://doi.org/10.1167/iovs.03-0559

Kilvington, S., & White, D. G. (1985). Rapid identification of thermophilic Naegleria, including Naegleria fowleri using API ZYM system. *Journal of Clinical Pathology*, *38*(11), 1289–1292. https://doi.org/10.1136/jcp.38.11.1289

Kim, B. R., Anderson, J. E., Mueller, S. a, Gaines, W. a, & Kendall, a M. (2002). Literature review — efficacy of various disinfectants against Legionella in water systems, *36*, 4433–4444.

Lau, H. Y., & Ashbolt, N. J. (2009). The role of biofilms and protozoa in legionella pathogenesis: Implications for drinking water. *Journal of Applied Microbiology*, *107*(2), 368–378. https://doi.org/10.1111/j.1365-2672.2009.04208.x

Locali, R. F., Almeida, M. De, & Oliveira-Júnior, I. S. De. (2006). Use of the histopathology in the differential diagnosis of drowning in fresh and salty water: an experimental model establishment in rats. *Acta Cirurgica Brasileira / Sociedade Brasileira Para Desenvolvimento Pesquisa Em Cirurgia*, *21*(4), 203–206. https://doi.org/10.1007/978-3-319-43449-0_3

Lopez, G. U., Gerba, C. P., Tamimi, A. H., Kitajima, M., Maxwell, S. L., & Rose, J. B. (2013). Transfer Efficiency of Bacteria and Viruses from Porous and Nonporous Fomites to Fingers under Different Relative Humidity Conditions, *79*(18), 5728–5734. https://doi.org/10.1128/AEM.01030-13

Lu, J., Buse, H. Y., Gomez-Alvarez, V., Struewing, I., Santo Domingo, J., & Ashbolt, N. J. (2014). Impact of drinking water conditions and copper materials on downstream biofilm microbial communities and Legionella pneumophila colonization. *Journal of Applied Microbiology*, *117*(3), 905–918. https://doi.org/10.1111/jam.12578

Ma, P., Visvesvara, G. S., Martinez, A. J., Theodore, F. H., Daggett, P. M., & Sawyer, T. K. (1990). Naegleria and Acanthamoeba Infections : Review. *Review of Infectious Diseases*, *12*(3), 490–513. https://doi.org/10.2307/4455558

Macler, B. A., & Regli, S. (1993). Use of Microbial Risk Assessment in Setting United-States Drinking-Water Standards. *International Journal of Food Microbiology*, *18*(4), 245–256.

https://doi.org/Doi 10.1016/0168-1605(93)90148-A

Marciano-Cabral, F., & Cabral, G. (2003). Acanthamoeba spp. as agents of disease in humans. *Clinical Microbiology Reviews*. https://doi.org/10.1128/CMR.16.2.273-307.2003

Mena, K. D., & Gerba, C. P. (2009). *Reviews of Environmental Contamination and Toxicology Vol 201* (Vol. 201). https://doi.org/10.1007/978-1-4419-0032-6

Miller, H. C., Wylie, J., Dejean, G., Kaksonen, A. H., Sutton, D., Braun, K., & Puzon, G. J. (2015). Reduced Efficiency of Chlorine Disinfection of Naegleria fowleri in a Drinking Water Distribution Biofilm. *Environmental Science and Technology*, *49*(18), 11125–11131. https://doi.org/10.1021/acs.est.5b02947

Moritz, M. M., Flemming, H. C., & Wingender, J. (2010). Integration of Pseudomonas aeruginosa and Legionella pneumophila in drinking water biofilms grown on domestic plumbing materials. *International Journal of Hygiene and Environmental Health*, *213*(3), 190–197. https://doi.org/10.1016/j.ijheh.2010.05.003

Mullis, S. N., & Falkinham, J. O. (2013). Adherence and biofilm formation of Mycobacterium avium, Mycobacterium intracellulare and Mycobacterium abscessus to household plumbing materials. *Journal of Applied Microbiology*, *115*(3), 908–914. https://doi.org/10.1111/jam.12272

National Research Council. (1999). *Setting Priorities for Drinking Water Contaminants*. Washington, DC: National Academies Press. https://doi.org/10.17226/6294

National Research Council. (2006). *Drinking Water Distribution Systems: Assessing and Reducing Risks*. Washington, DC: The National Academies Press. https://doi.org/10.17226/11728

Neuhäuser, M., & Hothorn, L. a. (1999). An exact Cochran–Armitage test for trend when dose–response shapes are a priori unknown. *Computational Statistics & Data Analysis*, *30*, 403–412. https://doi.org/10.1016/S0167-9473(98)00091-7

Nicas, M., & Best, D. (2008). A study quantifying the hand-to-face contact rate and its potential application to predicting respiratory tract infection. *Journal of Occupational and Environmental Hygiene*, *5*(6), 347–352. https://doi.org/10.1080/15459620802003896

Nicas, M., & Jones, R. M. (2009). Relative contributions of four exposure pathways to influenza infection risk. *Risk Analysis*, *29*(9), 1292–1303. https://doi.org/10.1111/j.1539-6924.2009.01253.x

O'Toole, J., Sinclair, M., Gibney, K., & Leder, K. (2015). Adoption of a microbial health-based target for australian drinking water regulation. *Journal of Water and Health*, *13*(3), 662–670. https://doi.org/10.2166/wh.2015.201

Ojielo, C. I., Cooke, K., Mancuso, P., Standiford, T. J., Olkiewicz, K. M., Clouthier, S., … Moore, B. B. (2003). Defective Phagocytosis and Clearance of Pseudomonas aeruginosa in the Lung Following Bone Marrow Transplantation. *The Journal of Immunology*, *171*(8), 4416–4424. https://doi.org/10.4049/jimmunol.171.8.4416

Papapetropoulou, M., Iliopoulou, J., Rodopoulou, G., Detorakis, J., & Paniara, O. (1994). Occurrence and Antibiotic-Resistance of Pseudomonas Species Isolated from Drinking Water in Southern Greece. *Journal of Chemotherapy*, *6*(2), 111–116.

Patrick, D. R., Findon, G., & Miller, T. E. (1997). Residual moisture determines the level of touch-contact-associated bacterial transfer following hand washing. *Epidemiology and Infection*, *119*(3), 319–325. https://doi.org/10.1017/S0950268897008261

Pearson, J. P., Feldman, M., & Iglewski, B. H. (2000). Pseudomonas aeruginosa Cell-to-Cell Signaling Is Required for Virulence in a Model of Acute Pulmonary Infection, *68*(7), 4331–4334.

Percival, S. L., & Walker, J. T. (1999). Potable water and biofilms: A review of the public health implications. *Biofouling*, *14*(2), 99–115. https://doi.org/10.1080/08927019909378402

Pitol, A. K., Bischel, H. N., Kohn, T., & Julian, T. R. (2017). Virus Transfer at the Skin-Liquid Interface. *Environmental Science and Technology*, *51*(24), 14417–14425. https://doi.org/10.1021/acs.est.7b04949

Poggio, E. C., Glynn, R. J., Schein, O. D., Seddon, J. M., Shannon, M. J., Scardino, V. A., & Kenyon, K. R. (1989). The incidence of ulcerative keratitis among users of daily-wear and extended-wear soft contact lenses. *The New England Journal of Medicine*, *321*(12), 779–783. https://doi.org/10.1056/NEJM199311113292002

Prest, E. I., Hammes, F., van Loosdrecht, M. C. M., & Vrouwenvelder, J. S. (2016). Biological stability of drinking water: Controlling factors, methods, and challenges. *Frontiers in Microbiology*, *7*(45), 1–24. https://doi.org/10.3389/fmicb.2016.00045

Raabe, O. G., Al-Bayati, M. A., Teague, S. V., & Rasolt, A. (1988). Regional Deposition of INhaled Monodisperse Coarse and Fine Aerosol Particles in Small Laboratory Animals. *Ann. Occup. Hyg.*, *32*.

Rhoads, W. J., Ji, P., Pruden, A., & Edwards, M. A. (2015). Water heater temperature set point and water use patterns influence Legionella pneumophila and associated microorganisms at the tap. *Microbiome*, *3*(1), 67. https://doi.org/10.1186/s40168-015-0134-1

Rogers J., Dowsett A.B., Dennis P.J., Lee J.V., & Keevil C.W. (1994). Influence of temperature and plumbing material selection on biofilm formation and growth of Legionella pneumophila in a model potable water system containing complex microbial flora. *Applied and Environmental Microbiology*, *60*(5), 1585–1592. Retrieved from http://ovidsp.ovid.com/ovidweb.cgi?T=JS&CSC=Y&NEWS=N&PAGE=fulltext&D=emed3&AN=1994137158

Rusin, P., Maxwell, S., & Gerba, C. (2002). Comparative surface-to-hand and fingertip-to-mouth transfer efficiency of gram-positive bacteria , gram-negative bacteria , and phage, 585–592.

Sadikot, R. T., Blackwell, T. S., Christman, J. W., & Prince, A. S. (2005). Pathogen-host interactions in pseudomonas aeruginosa pneumonia. *American Journal of Respiratory and Critical Care Medicine*, *171*(11), 1209–1223. https://doi.org/10.1164/rccm.200408-1044SO

Salehi, M., Abouali, M., Wang, M., Zhou, Z., Nejadhashemi, A. P., Mitchell, J., … Whelton, A. J. (2018). Case study: Fixture water use and drinking water quality in a new residential green building. *Chemosphere*, *195*, 80–89. https://doi.org/10.1016/j.chemosphere.2017.11.070

Schoen, M. E., & Ashbolt, N. J. (2011). An in-premise model for Legionella exposure during showering events. *Water Research*, *45*(18), 5826–5836. https://doi.org/10.1016/j.watres.2011.08.031

Schuster, F. L., & Visvesvara, G. S. (2004). Amebae and ciliated protozoa as causal agents of waterborne zoonotic disease. *Veterinary Parasitology*, *126*, 91–120. https://doi.org/10.1016/j.vetpar.2004.09.019

Schuster, M., Sexton, D. J., Diggle, S. P., & Greenberg, E. P. (2013). Acyl-Homoserine Lactone Quorum Sensing : From Evolution to Application. https://doi.org/10.1146/annurev-micro-092412-155635

Shoff, M. E., Rogerson, A., Kessler, K., Schatz, S., & Seal, D. V. (2008). Prevalence of Acanthamoeba and other naked amoebae in South Florida domestic water. *Journal of Water and Health*, *6*(1), 99–104. https://doi.org/10.2166/wh.2007.014

Snary, E. L., Swart, A. N., Simons, R. R. L., Rita, A., Domingues, C., Vigre, H., … Hill, A. A. (2016). A Quantitative Microbiological Risk Assessment for Salmonella in Pigs for the European Union, *36*(3). https://doi.org/10.1111/risa.12586

Sotoyama, M., Villanueva, M., Jonai, H., & Saito, S. (1995). Ocular Surface Area as an Informative Index of Visual Ergonomics. *Industrial Health*, *33*, 43–56.

Stockman, L. J., Wright, C. J., Visvesvara, G. S., Fields, B. S., & Beach, M. J. (2011). Prevalence of Acanthamoeba spp. and other free-living amoebae in household water, Ohio, USA - 1990-1992. *Parasitology Research*, *108*(3), 621–627. https://doi.org/10.1007/s00436-010-2120-7

Streeter, K., & Katouli, M. (2016). Pseudomonas aeruginosa: A review of their Pathogenesis and Prevalence in Clinical Settings and the Environment. *Infection, Epidemiology and Medicine*, *2*(1), 25–32. https://doi.org/10.18869/modares.iem.2.1.25

Tamrakar, S. (2013). Pseudomonas aeruginosa (Contact lens): Dose Response Models. Retrieved

March 12, 2019, from http://qmrawiki.canr.msu.edu/index.php/Pseudomonas_aeruginosa_(Contact_lens):_Dose_Response_Models

Tang, H. B., Mango, E. D. I., Bryan, R., Gambello, M., Iglewski, B. H., Goldberg, J. B., & Prince, A. (1996). Contribution of Specific Pseudomonas aeruginosa Virulence Factors to Pathogenesis of Pneumonia in a Neonatal Mouse Model of Infection, *64*(1), 37–43.

Teunis, P. F. M., & Havelaar, A. H. (2000). The Beta Poisson dose-response model is not a single-hit model. *Risk Analysis*, *20*(4), 513–520. https://doi.org/10.1111/0272-4332.204048

Thomas, J. M., & Ashbolt, N. J. (2010). Do Free-Living Amoebae in Treated Drinking Water Systems Present an Emerging Health Risk? *Environmental Science & Technology*, *45*(3), 860–869. https://doi.org/10.1021/es102876y

Thomas, V., Blanc, D., Bille, J., & Greub, G. (2005). Biodiversity of Amoebae Resistant Micro-Organisms in an Hospital Water Network. *Abstracts of the Interscience Conference on Antimicrobial Agents and Chemotherapy*, *45*(4), 318. https://doi.org/10.1128/AEM.72.4.2428

Torres, A., Serra-batlles, J., Ferrer, A., Jimenez, P., Celis, R., Cobo, E., & Rodriguez-roisin, R. (1991). Severe Community-acquired Pneumonia. *The American Review of Respiratory Disease, 144*, 312–318.

Trautmann, M., Lepper, P. M., & Haller, M. (2005). Ecology of Pseudomonas aeruginosa in the intensive care unit and the evolving role of water outlets as a reservoir of the organism. *American Journal of Infection Control*, *33*(5 SUPPL. 1), 41–49. https://doi.org/10.1016/j.ajic.2005.03.006

US Environmental Protection Agency. (1998). Announcement of the Drinking Water Contaminant Candidate List. *Federal Register, 63*(40), 10274–10287.

US Environmental Protection Agency. (2005). Drinking Water Contaminant Candidate List 2; Final Notice. *Federal Register*, *70*(36), 9071–9077. https://doi.org/10.1017/cbo9781107337442.008

US Environmental Protection Agency. (2009). Drinking Water Contaminant Candidate List 3-Final. *Federal Register*, *74*(194), 51850–51862.

US Environmental Protection Agency. (2011). Exposure Factors Handbook: 2011 Edition. *U.S. Environmental Protection Agency*, *EPA/600/R*-(September), 1–1466. https://doi.org/EPA/600/R-090/052F

US Environmental Protection Agency. (2016). Drinking Water Contaminant Candidate List 4-Final. *Federal Register, 81*(222), 81099–81114.

Van Der Merwe, V., Duvenage, S., & Korsten, L. (2013). Comparison of biofilm formation and water quality when water from different sources was stored in large commercial water storage tanks. *Journal of Water and Health*, *11*(1), 30–40. https://doi.org/10.2166/wh.2012.014

van der Wielen, P. W. J. J., & van der Kooij, D. (2013). Nontuberculous mycobacteria, fungi, and opportunistic pathogens in unchlorinated drinking water in the Netherlands. *Applied and Environmental Microbiology*, *79*(3), 825–834. https://doi.org/10.1128/AEM.02748-12

Vela, A. I., Gracía, E., Fernández, A., Domínguez, L., & Fernández-Garayzábal, J. F. (2006). Isolation of Corynebacterium xerosis from animal clinical specimens. *Journal of Clinical Microbiology*, *44*(6), 2242–2243. https://doi.org/10.1128/JCM.02473-05

Wang, H., Edwards, M., Falkinham, J. O., & Pruden, A. (2012). Molecular survey of the occurrence of legionella spp., mycobacterium spp., pseudomonas aeruginosa, and amoeba hosts in two chloraminated drinking water distribution systems. *Applied and Environmental Microbiology*, *78*(17), 6285–6294. https://doi.org/10.1128/AEM.01492-12

Weir, M. H., Mitchell, J., Flynn, W., & Pope, J. M. (2017). Development of a microbial dose response visualization and modelling application for QMRA modelers and educators. *Environmental Modelling and Software*, *88*, 74–83. https://doi.org/10.1016/j.envsoft.2016.11.011

Whiley, H., Keegan, A., Giglio, S., & Bentham, R. (2012). Mycobacterium avium complex - the role of potable water in disease transmission. *Journal of Applied Microbiology*, *113*(2), 223–232. https://doi.org/10.1111/j.1365-2672.2012.05298.x

Wingender, J., & Flemming, H. (2011a). International Journal of Hygiene and Biofilms in drinking water and their role as reservoir for pathogens. *International Journal of Hygiene and Environmental Health*, *214*(6), 417–423. https://doi.org/10.1016/j.ijheh.2011.05.009

Wingender, J., & Flemming, H. C. (2011b). Biofilms in drinking water and their role as reservoir for pathogens. *International Journal of Hygiene and Environmental Health*, *214*(6), 417–423. https://doi.org/10.1016/j.ijheh.2011.05.009

World Health Organization. (2007). *Legionella and the Prevention of Legionellosis*. *World Health Orgranization*. https://doi.org/10.3201/eid1406.080345

Wu, H., Song, Z., Hentzer, M., Andersen, J. B., Molin, S., Givskov, M., & Høiby, N. (2004). Synthetic furanones inhibit quorum-sensing and enhance bacterial clearance in Pseudomonas aeruginosa lung infection in mice, *53*(6), 1054–1061. https://doi.org/10.1093/jac/dkh223

Xie, G., Roiko, A., Stratton, H., Lemckert, C., Dunn, P. K., & Mengersen, K. (2017). Guidelines for Use of the Approximate Beta-Poisson Dose–Response Model. *Risk Analysis*, *37*(7), 1388–1402. https://doi.org/10.1111/risa.12682

Yoder, J. S., Verani, J., Heidman, N., Hoppe-Bauer, J., ALfonso, E. C., Miller, D., … Beach, M. J. (2012). Acanthamoeba keratitis: The Persistence of Cases Following a Multistate Outbreak. *Ophthalmic Epidemiology*, *19*(4), 221–225. https://doi.org/10.3109/09286586.2012.681336

Yoon, S. H., & Lee, J. (2016). Computing the surface area of three-dimensional scanned human data. *Symmetry*, *8*(7), 1–14. https://doi.org/10.3390/sym8070067

Zaied, R. A. (2017). Water use and time analysis in ablution from taps. *Applied Water Science*, *7*(5), 2329–2336. https://doi.org/10.1007/s13201-016-0407-2

Zheng, M., He, C., & He, Q. (2015). Fate of free chlorine in drinking water during distribution in premise plumbing. *Ecotoxicology*, *24*(10), 2151–2155. https://doi.org/10.1007/s10646-015-1544-3