March 2018

Effectiveness of Biocide Substitution and Management Plan Implementation for the Control of

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Effectiveness of Biocide Substitution and Management Plan Implementation for the Control of *Legionella pneumophila* in Cooling Tower Waters

by

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A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science in Public Health
with a concentration in Industrial Hygiene
Department of Environmental and Occupational Health
College of Public Health
University of South Florida

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Date of Approval:
February 28, 2018

Keywords: Industrial Hygiene, Bacteria, Legionella, Cooling Towers, Biocides

Dedication

This thesis is dedicated to my mother, brother, aunt, and grandmother in heaven: Iris M. Gonzalez Martinez, Orlando X. Sanchez Gonzalez, Nancy E. Gonzalez Martinez, and Maria A. Martinez Liquet. I could not have accomplished any of this without your support and steadfast belief in me.

Acknowledgments

I would like to thank all the faculty and administrative personnel at the University of South Florida College of Public Health, Department of Environmental Health and Safety. Also, I would like to acknowledge CDC/NIOSH (T42-008438) for providing the funding for my education at USF.

Table of Contents

List of Tables

List of Abbreviations and Acronyms

ACGIH	American Conference of Governmental Industrial Hygienists
CFU	Colony Forming Unit
LP	*Legionella pneumophila*
LD	Legionnaires' Disease
NIOSH	National Institutes for Occupational Safety and Health
OSHA	Occupational Safety and Health Administration
ASHRAE	American Society of Heating, Refrigeration and Air-Conditioning Engineers
PEL	Permissible Exposure Limit
TLV	Threshold Limit Value
CDC	Centers for Disease Control and Prevention
CTS	Cooling Tower Site

Abstract

After the notorious outbreak and discovery of *Legionella* bacteria in 1976, the waterborne pathogen was added to the list of disease-causing agents associated with the built environment. *Legionella pneumophila* was discovered when it was identified as the agent that caused 34 deaths and an outbreak of pneumonia-like symptoms in several attendees of the 1976 American Legion Convention held in Philadelphia (OSHA, 2017).

Recently published data from the year 2015 reported more than 6,000 Legionnaires' cases identified in the United States (CDC, 2016). This is a concerning number given that one in every ten infected persons will die of the disease. It is believed that case numbers are likely under-reported, given that Legionnaires' disease is very difficult to diagnose.

Legionella species live naturally in bodies of water, including lakes and rivers. Legionnaires' disease has been associated with the introduction of *Legionella* into manmade water systems. The presence of *Legionella* has been reported in cooling towers, domestic hot-water systems, humidifiers, decorative fountains, grocery spray misters, spas, whirlpools, and dental water lines, among other systems housing stagnant water (CDC, OSHA, 2017). From an occupational exposure standpoint, cooling towers are considered the most concerning source of *Legionella pneumophila* exposures, based on data from previous cases (Principe et al., 2017).

The purpose of this research was to measure the effectiveness of biocide substitution and maintenance management in evaporative condensers. Such condensers were previously identified as having high counts of *Legionella pneumophila* in the water and/or on surfaces. The study sites were in the states of Florida and Georgia. Initial water testing for *Legionella* was carried out between July and August of 2016. Results from 2016 showed high counts of colony forming units (CFU) per millimeter (mL) at baseline assessment. An intervention of biocide substitution and enhanced management planning was recommended to lower or eliminate *L. pneumophila* from the water basins of the evaporative condensers. Follow-up results of water sampling conducted between July and August 2017 showed reduction of CFU counts after the intervention plan had been implemented for an entire year.

Introduction and Background

After its discovery in 1976, *Legionella pneumophila* rapidly became a significant public health concern present in the built environment. *Legionella* is a gram-negative bacterium that occurs naturally in freshwater bodies. The cell sizes are less than 1 micrometer wide and less than 3 micrometers long. This bacterium is an aerobic bacillus with a single polar flagellum, and it does not form spores. It successfully replicates freely in water and inside eukaryotic cells. *Legionella* prefers warm to hot temperatures in which to replicate, with optimal growth temperatures found to be between 35–40°C. Although the bacterium is aerobic, it requires only a very small amount of oxygen, explaining its ability to replicate inside eukaryotic cells.

Legionnaires' disease (LD) and Pontiac fever (PF) are the infections caused by *L. pneumoohila*. Discovered in Pontiac, Michigan, PF is a flulike, pneumonia illness. Due to poor consensus and clinical definition, PF cases seem rare and are presumably underreported (Prussin et al., 2007). LD is defined as an advanced form of PF. The organism incubation period ranges from about two to fourteen days, and the infection colonizes primarily the alveolar macrophages. The route of exposure has been shown to be inhalation. There has not been a case reported in which the infection has been contracted from an infected person. Common symptoms of LD include headache, cough, shortness of breath, muscle aches, vomiting, and diarrhea.

Legionella outbreaks occur in many countries around the world. Industrialized cities have a tendency of reporting a higher number of cases as a result of a higher number of buildings and their engineered water systems (Principe et al, 2007; Prussin et al, 2017). From its discovery at the 1976 American Legion Convention in Philadelphia, *Legionella* has been considered an important public health concern of the built environment. From this outbreak, a group of attendees reported pneumonia symptoms and more than 20 died. After scientists investigated this outbreak they discovered *Legionella* and tracked its sources to the cooling towers system. After this important and well-known outbreak, outbreaks started to be reported along the US and many other countries. Among other significant outbreaks is an office building in New York City in 1984 affecting more than 60 people; an industrial engineering plant in Bolton, United Kingdom, affecting more than 40 people; an adult nursing home in Scarborough, Canada, affecting more than 30 people; and most recently, an outbreak at Las Vegas resort affecting two hotel guests and resulting in evacuation of several floors.

Legionella is a problem in the built environment due to its ability to grow in the warm to hot temperatures at which many engineered water systems operate. Its presence becomes a risk for building occupants. The bacterium has been found to successfully colonize whirlpools, water fountains, showers, and cooling towers, among other locations. However, it has been found that among all the possible sources for *Legionella* in the built environment, cooling towers tend to top the list (Kim et al., 2014; Rangel et al., 2011; Principe et al., 2017; Li et al., 2015; Luksamijaruku et al., 2014). The significance of cooling towers as a source of *Legionella* in the built environment comes from the temperature needed to maintain their proper functioning. As estimated by manufacturers and based upon average sales by year, there are approximately 17,500 cooling towers currently in use in North America. It is also estimated that approximately 250,000 cooling towers are manufactured annually for national and international shipment (Rangel et al., 2011). A cooling tower's operational water temperature range is estimated to be from 78°F (25.6°C) to 95°F (35°C). The temperature range for cooling towers makes them ideal for *Legionella* amplification, with an optimal temperature range of 77°F (25°C) to 113°F (45°C) (Wadowsky et al, 1985; Lin et al., 2008). Another beneficial factor for replication in cooling towers is the presence of iron, a construction material in cooling towers that is necessary for *Legionella* growth (Hoffman, 2008). As previously mentioned, *Legionella* successfully colonizes eukaryotic cells, specifically amoeba. In cooling towers, amoeba buildup on surfaces is a problem, as it gives *Legionella* a shielded environment for growth. The epidemiological data for *Legionella* outbreaks and sample monitoring results often point to cooling towers as the primary source for *Legionella* infection and have found high levels of CFU within water samples.

Legionnaires' disease is considered a very common waterborne pathogenic disease. According to the U.S. Centers for Disease Control and Prevention (CDC), about 6,000 cases were reported during 2015 in the United States. In 2014, the European Union reported a higher number, close to 7,000 cases, according to the European Centre for Disease Prevention and Control (ECDC). It is believed that these numbers may be underestimated, as the symptoms can be easily confused with those of the common flu. Epidemiological data indicate that at least one in ten infected persons will die from LD (Correia et al., 2016).

Legionella pneumophila is a biological agent, and like many of them, it does not have established regulatory limits for exposure, although guidelines for CFU counts in water have been recommended by various agencies. The CDC and ECDC consider LD a notifiable disease, therefore clinicians must report cases as diagnosed. The CDC has published guidance aimed specifically at building owners and managers that may not have extensive technical knowledge to help them develop a water management plan for preventing *Legionella* growth in water systems.

Due to *Legionella*'s presence in hotels, office buildings, and hospitals, among many other facilities where employees are at risk, the Occupational Safety and Health Administration (OSHA) has a technical manual for employers to help them create a management plan that includes employee awareness, monitoring, and recordkeeping for *Legionella* in the workplace. The OSHA publication contains recommended actions based on the CFU counts of collected water samples. According to OSHA, detection of Legionella within a sampled water system at 100 CFU/ml is classified as 'Action-1', which triggers "prompt cleaning and/or biocide treatment". Similarly, detection of Legionella at 1000 CFU/ml is classified as 'Action-2', triggering "immediate cleaning and/or biocide treatment", and "prompt steps to prevent employee exposure". The American Society of Heating, Refrigerating and Air-Conditioning Engineers (ASHRAE) also published guidelines for *Legionella* control in water systems; this guide delineates how to recognize a facility's risk for *Legionella* presence in the water systems, how to prevent it, and how to control it if detected. ASHRAE recommends maintaining *Legionella* levels at or below 250 CFU/mL. The State of New York has also created an in-depth program to monitor and prevent LD cases, and this effort specifically addresses cooling towers. Building owners who have cooling towers within their facilities are required to list their towers in a registry and must submit specific machinery information, maintenance schedules, and *Legionella* monitoring results. The Florida Department of Health adopted the recommendations published by OSHA, requiring the state to follow the recommended actions for *Legionella* detection.

This study provided an opportunity to evaluate the efficacy of a consistent biocide application schedule and enhanced management plan for Legionella prevention. The four research questions of the study are as follows:

1- Does routine biocide application improve *Legionella* control in cooling towers based on water monitoring performed within a one-year span?
2- Does adherence to a *Legionella* maintenance management program improve *Legionella* control in cooling towers?
3- Does geographic location of a cooling tower influence the outcome of routine biocide application and adherence to a management program?
4- Does the intended use of a cooling tower site's facility have an effect on *Legionella* counts?

The University of South Florida's Institutional Review Board (IRB) classified this study as a program evaluation, and determined there was no intervention with human subjects, thus waiving IRB requirements.

Literature Review

The lungs, one of the organs of the respiratory system, are vital for human survival. This organ is also extremely susceptible to entry of xenobiotics due to its connection to body cavities such as the nose and mouth. The air humans breathe may be contaminated with multiple agents, including gases, particles, fungi, and bacteria. The aerodynamic size of the object entering the respiratory system is a determinant where contaminants may be deposited (Plog, 2002). In the case of *Legionella pneumophila*, a bacterial agent, its small size helps the colonization go deep into the lungs' alveolar region. As a result, *L. pneumophila* may cause respiratory illness and in some cases death (Hoffman, 2008).

The risk associated with being exposed to *Legionella* and contracting Legionnaires' disease or Pontiac fever is well documented within the literature (Principe et al, 2017; Macher 1999). This topic has been studied from different professional viewpoints, including those of industrial hygienists, engineers, facility managers, and the medical community. As more information becomes available, different sectors are working together to provide a clearer picture of how to prevent, evaluate, and control *Legionella* in the built environment.

Legionella presents a public health threat given its success in colonizing water systems (CDC 2017). Water, composed of a hydrogen atom and two oxygen atoms, is a vital substance for human life, not only for human consumption but also many other human activities, such as cooking, sanitary services, and cleaning, among others. Most daily human activities take place in buildings where engineered water systems are installed to support such activities. Generally, engineered water systems are kept at 140°F (60°C) and circulated at temperatures above 122°F (50°C). *Legionella* will multiply at temperatures between 68°F (20°C) and 122°F (50°C) (OSHA, 2007). It is for this reason that preventative measures and constant monitoring plays an important role in preventing *Legionella* growth and potential risk of exposure.

LD and PF are recognized and monitored by the CDC, and OSHA also recognizes the risk for employees to be exposed and infected with *Legionella*. From a public health perspective, the CDC has also initiated an awareness program for the public and facility managers. In a series of publications, the CDC offers current statistics for the disease and patterns for how *Legionella* can colonize the built environment and put building occupants at risk of contracting LD or PF (CDC, 2017). From the occupational perspective, OSHA has published directives for awareness of possible *Legionella* exposure in the workplace for many years. In a technical manual published January 20, 1999, the agency described possible *Legionella* reservoirs in the workplace and also described how industrial hygienists should apply preventive measures, hazard recognition, evaluation, and control on this matter (OSHA, 2017).

Within the technical sector, ASHRAE has published two editions of *Legionellosis: Risk Management for Building Water Systems*, which presents guidelines for identifying, evaluating, and controlling *Legionella* in engineered water systems. This publication is one of the most helpful in the United States and was intended to set the standard for the recognition, evaluation and control of *Legionella* by the technical community. ASHRAE recommends that *Legionella* levels be kept below 250 CFU/mL; above this level it is believed that *Legionella* presents risk of exposure from water systems. In this publication, ASHRAE offers guidance for managing the problem via biocide application, water rotation (periodic draining of the water system), temperature monitoring, and specific design recommendations from engineers.

Efforts have been made to promulgate preventive measures and management recommendations for the *Legionella* problem. In the article 'Controlling Legionnaire's Disease' published in the journal *Indoor and Built Environment* (2003), author Brundrett outlines four preventive measures that should be taken into consideration by building engineers and facility managers. In this article author Brundrett proposes methods to prevent the reproduction of *Legionella* to levels that could potentially infect people. The first method requires limiting water storage in systems for no more than a full working day. The second method is to keep water from escaping the system. In cooling towers, this can be accomplished by using a high-drift eliminator that will lower or eliminate the escape of water mist. The third prevention method requires lowering or eliminating the possibility of aerosols from coming into contact with people; this can be done by planning the location of the cooling tower and the position of the tower's entry point for air intake. The fourth and final prevention method addresses the protection of susceptible people, by controlling the contact of the cooling tower water with smokers, individuals over 50 years old, and those with existing medical conditions.

A study carried out among 22 factories in China (Li et al., 2015) tested the cooling towers for *Legionella* contamination. In this study 255 industrial cooling tower water samples were collected, and 37.5% tested positive for *Legionella*. Samples were collected monthly for a full year. *Legionella* levels averaged 9.1 CFU/mL among all collected samples within the 22 factories. The highest levels were detected in the summer and autumn and the lowest in Spring. The investigators discussed in their findings that higher recorded air temperatures in summer and autumn contribute positively to *Legionella* reproduction.

A recent epidemiological study (Principe et al., 2017) reviewed a compilation of published outbreaks information. By reviewing the published outbreaks information, the authors of this epidemiological study consider the different characteristics including number of patients, number of deaths, source of exposure and etiology of the organism at which the exposure occurred. In this study of 47 selected outbreaks, 39 were directly related to the occupational setting, with the remaining classified as community-based outbreaks. Another interesting finding was that of the 47 outbreaks selected,19 originated from cooling towers.

A systematic review of the maintenance of evaporative cooling systems by Rangel et al. 2010, aimed to study compliance with maintenance guidelines provided by the scientific community and authoritative agencies. In this study, high levels of discrepancy were found in how evaporative cooling tower maintenance programs were conducted for the control of *Legionella* growth. Although it was found that there is agreement with published guidelines regarding the need for biocide application, prevention of stagnant water, testing for the presence of Legionella, installation of drift eliminators, and implementation of general inspections with a documented maintenance plan to avoid *Legionella* replication, there were discrepancies in the frequency of maintenance components. This study also compared information from outbreaks against the frequency of adherence to maintenance protocols to research the validity of recommended guidelines and identify the characteristics of those cooling towers that had an outbreak. The authors concluded that inconsistencies to published guidelines could result in misunderstandings among personnel charged with maintenance of cooling towers. Also, combinations of maintenance practices may be leading to *Legionella* growth and possible outbreaks. From this study, it was concluded that there must be agreement within the guidelines and such recommendations should be written according to agreed-upon scientific data.

Methods

Study Site Selection

Eleven cooling tower sites (CTS) were evaluated for the presence of *Legionella*. Nine of the eleven were located between Central Florida and Northeast Florida. The remaining two sites were in the greater Atlanta Georgia area. Of the eleven cooling tower sites, a total of 35 condensers were tested. Initial sampling was conducted during the months of June and July 2016 and follow-up sampling was conducted during the months of July and August 2017. A comparison was made between the geographical location and the *Legionella* levels at each sampling event independently. The intended use of the facility was also compared for the predisposition for *Legionella* detection. A listing of each CTS and relevant details are provided in Table I.

TABLE I. Cooling tower site geographical locations, facility intended use, evaporative condenser identification, and sampling event dates

Cooling Tower Site ID (CTS)	Facility Intended Use	Condenser ID	Geographical Region	Baseline Sampling Month and Year (MM/YY)	Follow-Up Sampling Month and Year (MM/YY)
CTS-1	Food Manufacturing & Packaging	EC-01	West Central Florida	Jun-16	Jul-17
		EC-02			
CTS-2	Food Manufacturing & Packaging	EC-03			
		EC-04			
CTS-3	Food Manufacturing & Packaging	EC-05			
CTS-4	Food Manufacturing & Packaging	EC-06			
CTS-5	Food Manufacturing & Packaging	EC-07			
CTS-6	Food Manufacturing & Packaging	EC-08			
CTS-7	Warehouse	EC-09	East Atlanta	Jul-16	
		EC-10			
		EC-11			
		EC-12			
		EC-13			
		EC-14			
		EC-15			
		EC-16			
CTS-8	Food Manufacturing & Packaging	EC-17			
		EC-18			
CTS-9	Warehouse	EC-19	West Atlanta		
		EC-20			
		EC-21			

TABLE I. Continued

Cooling Tower Site ID (CTS)	Facility Intended Use	Condenser ID	Geographical Region	Baseline Sampling Month and Year (MM/YY)	Follow-Up Sampling Month and Year (MM/YY)
CTS-10	Warehouse	EC-22	Central Florida	Jun-16	Jul-17
		EC-23			
		EC-24			
		EC-25			
		EC-26			
		EC-27			
		EC-28			
		EC-29			
CTS-11	Warehouse	EC-30	Northeast Florida		
		EC-31			
		EC-32			
		EC-33			
		EC-34			
		EC-35			

Sample Collection

Composite grab samples of the basin waters were collected following procedures recommended by the International Organization of Standardization (ISO 11731:2017). Sample collection containers were obtained from a contract laboratory, and each sample container was labeled with a unique identifier and handled in such a manner as to prevent cross-contamination between samples. A total of 1,000 mL of water was collected from each evaporative condenser sampled. All samples submitted for analysis were collected, transported, and shipped to the contract laboratory in insulated containers. A nationally recognized laboratory was chosen as the contract laboratory based on their accreditation with the CDC ELITE Program, which certifies knowledge and experience in the analysis of biological materials.

Biocide Application and Management Plan Assessment

Before this intervention, the cooling towers' maintenance consisted of cleaning and biocide treatment. Cleanings were scheduled every six months. Biocide application was encouraged but not recorded or documented at the respective CTS. Table II presents the five biocides used among all cooling tower sites. Prior to this study, an indiscriminate selection and application of the available biocides was used without consensus and recordkeeping of date of application and type of biocide applied.

TABLE II. Biocide use details through July 2016

Biocide Identification	Biocide Active Ingredient(s)	Percentage per Solution (%)
B1	Glutaldehyde	50
B2	Carboxylic acid aromatic amine	5–10
B3	Tetrasodium HEDP Sodium tolytriazole Sodium hydroxide	5–10 5–10 1–5
B4	Glutaldehyde	15
B5	Sodium hypochlorite Sodium bromide Sodium hydroxide	3.365 9.23 1–10

After the 2016 sampling event, all cooling tower sites agreed to be treated with one biocide, selected according to the active ingredient and the concentration per solution. As part of the treatment an independent, professional contractor performed a disinfection cleaning. The site personnel also replaced cooling tower parts that had been greatly affected by biofilm. In other areas where biofilm buildup was observed, intense scrubbing was recommended. Also, a minor inspection and cleaning took placed every three months. Date and observations from quarterly inspection/cleaning were recorded. It should be noted that all cooling tower sites were managed by the same team, therefore a homogenous application of the practices was assumed.

Data Statistical Test

The paired sample t-test was selected to determine statistical difference between the two observations measured in the same evaporative condensers at different times (Sullivan, 2011). The t-test was conducted between differences between the sample levels in 2016 and 2017. Only condensers with levels above 0 CFU/mL in both sampling events 2016 and 2017 where included in this test (N=8). Statistically significant difference between both data points is concluded with a p-value of less than 5%.

Results

The *Legionella pneumophila* sampling results for 2016 and 2017 sampling events are presented in Table III. Results are presented for all 35 evaporative condensers located among 11 cooling tower sites. During the initial sampling event in 2016, the highest *Legionella* level of 4,160 CFU/mL was detected in EC-02, located at CTS-1, and the lowest detectable level of 20 CFU/mL was in EC-25, located at CTS-10. The average level per evaporative condenser for the 2016 sampling event was calculated to be 357 CFU/mL. No *Legionella* was detected during the 2016 sampling event for evaporative condensers located at CTS-7, CTS-9, and CTS-11.

The second sampling event, in July 2017 found the highest *Legionella* level detected was 13.2 CFU/mL in EC-05, located at CTS-3, and the lowest level was 0.29 CFU/mL in EC-23 and EC-29, both located at CTS-10. The average *Legionella* level for 2017 was calculated to be 1.90 CFU/mL. No levels were detected in any of the evaporative condensers located at CTS-7, CTS-8, CTS-9, and CTS-11. Between 2016 and 2017, a 90% reduction in average levels was found.

TABLE III. – *Legionella pneumophila* Levels by Cooling Tower Site

Cooling Tower Site (CTS)	Condenser ID	2016 *Legionella pneumophila* Levels (CFU/mL)	2017 Follow-Up *Legionella pneumophila* Levels (CFU/mL)
CTS-1	EC-01	3900	6.35
	EC-02	4160	2.5
CTS-2	EC-03	40	8.66
	EC-04	1380	8.57
CTS-3	EC-05	1840	13.2
CTS-4	EC-06	380	8.62
CTS-5	EC-07	80	11
CTS-6	EC-08	40	7.17
CTS-7	EC-09	ND	ND
	EC-10	ND	ND
	EC-11	ND	ND
	EC-12	ND	ND
	EC-13	ND	ND
	EC-14	ND	ND
	EC-15	ND	ND
	EC-16	ND	ND
CTS-8	EC-17	80	ND
	EC-18	560	ND
CTS-9	EC-19	ND	ND
	EC-20	ND	ND
	EC-21	ND	ND
CTS-10	EC-22	ND	ND
	EC-23	ND	0.29
	EC-24	ND	ND
	EC-25	20	ND
	EC-26	ND	ND
	EC-27	ND	ND
	EC-28	ND	ND
	EC-29	ND	0.29
CTS-11	EC-30	ND	ND
	EC-31	ND	ND
	EC-32	ND	ND
	EC-33	ND	ND
	EC-34	ND	ND
	EC-35	ND	ND

ND= None Detected

The paired t-test between the eight evaporative condensers that comply with the selective criteria showed significant reduction in Legionella levels. Table IV presents the test result.

TABLE IV. Paired T-Test for Selected *Legionella* Levels

Statistical Test	Sample Size (N)	p-value(%)
Paired T-Test	8	4.57

Discussion

The purpose of this research was to determine whether the application of biocide and the implementation of a *Legionella* maintenance management plan could reduce *Legionella* levels. This study also allowed evaluation of whether a cooling tower's geographic location and a facility's intended use play a role in *Legionella* counts. By comparison the results obtained from 2016 and 2017 sampling events showed reduction in the presence of *Legionella* in those evaporative condensers with detection at 2016. Among the sites that had no *Legionella* detected in 2016, only two evaporative condensers—both located at CTS-10—had detectable levels in 2017, where previously there had been none.

The evaporative condensers associated with CTS-1, CTS-2, CTS-3, CTS-4, CTS-5, CTS-6, CTS-8, and CTS-10 with detected levels in 2016 showed significant reduction or no detectable levels in the follow-up samplings in 2017. This reduction suggests that biocide application with supporting documentation, and a maintenance management plan, help reduce *Legionella* levels in cooling towers. Standard work practices guidelines recommend maintaining levels at or below 250 CFU/mL (ASHRAE, 2015). During the 2016 sampling event, 31% of the evaporative condensers showed the presence of *Legionella*, and 17% of those were above the levels recommended by ASHRAE. OSHA and the Florida Department of Health recommend prompt cleaning and biocide treatment for water systems with levels at or above 100 CFU/mL and immediate cleaning and biocide treatment for water systems with levels at or above 1,000 CFU/mL. Thus, results from the 2016 sampling event showed that 17% of the evaporative condensers needed immediate action taken.

After the 2016 sampling event, an analysis of the findings was performed, and an industrial hygiene intervention was proposed to the facility managers. This intervention recommended the use and documentation, of B1 across the 11 cooling tower sites, mechanical check every 30 days, and a cleaning every 90 day.

Cooling tower sites CTS-7, CTS-9, and CTS-11 had no detection in 2016 and in 2017. Informal conversations with the local cooling tower sites' personnel revealed that application of biocide was occurring continuously, performing cleaning and documenting all actions taken according to the schedule proposed during the intervention.

From a biocide application perspective, its application is a positive tool in controlling *Legionella* in building water systems, including cooling towers (CDC, 2017; OSHA, 2017; ASHRAE 2015; Rangel et al., 2011; Hutchler 2000; Forstmrirt et al., 2005; Luksamijarulkul et al., 2014; Flannery et al., 2006). Prior to the 2016 sampling event the biocide application was not being documented, therefore there was no evidence that the evaporative condensers that showed *Legionella* detection had been treated with biocide. After a year of constant B1 application, the most concentrated biocide available for these facilities, a significant reduction in Legionella was observed. For example, the highest level was detected in EC-02 (4,160 CFU/mL), located at CTS-1 during the 2016 sampling event. After routine biocide application of B1 for a year, results for 2017 EC-02 levels were 2.5 CFU/mL, a reduction of 99%. Prior to 2016, biocide was injected directly into the water line that travels to the evaporative condensers, but only when workers remembered to connect it and the frequency was unknown. For 2017, the substitution and application of B1 only was conducted in conjunction with a 30-day check of both biocide tank levels and of the pump that flows the biocide into the water line to ensure it was working properly. The substitution form of control accompanying the biocide application complies with the control hierarchy for application effectiveness in industrial hygiene, as cited in important industry textbooks such as *The Occupational Environment (2003)* and *Fundamentals of Industrial Hygiene (2002)*.

From a management program perspective, the management team should start with the assumption that *Legionella* is present at very low levels in the cooling tower waters (Brundrett, 2003). Based on this assumption, administrative controls are necessary in addition to preventive design measures for towers, application of biocide, and controlled temperature of the water (Brundrett, 2003; Luksamijarulkul et al., 2014). Facility management and workers, familiar with cooling towers systems, were informed of the presence of *Legionella* in the affected cooling tower sites, and as a result, they agreed to participate by following the maintenance schedules to control the bacteria. Employee involvement and training are administrative controls that help the reduction of accidents and illness at the workplace (DiNardi, 2003). For that reason, the workers were educated as to their risk and trained in how to identify possible signs of *Legionella*, such as biofilm accumulation, turbid water, and optimal temperature range in the evaporative condensers. The workers were informed of the biocide selection and the recording process for its continuous application. Every 30 days, a general inspection of the cooling towers' evaporative condensers was conducted, a practice that complies with the majority of standard work practices for *Legionella* control in cooling towers (Rangel et al., 2011). In addition, every 90 days a cleaning was conducted by the same workers, and any parts with visible biofilm were replaced with new parts. The overall reduction of *Legionella* levels directly correlated with the maintenance practices followed from July 2016 to July 2017. Prompt action by the workers monitoring the cooling towers avoided the favorable conditions for *Legionella* growth. The implementation of this practice aligns with recommendations by the technical sector and government public health agencies (ASHRAE, 2015; CDC, 2017; OSHA, 2017). Where evaporative condensers did not present *Legionella*, an unofficial management plan was being followed and no change was recommended there was no detection in 2017, with the exception of EC-23 and EC-29, both located at CTS-10. The explanation for this relies on the assumption that *Legionella* is ubiquitous, and a few microbes are always present in a given system.

The geographical region may not play a role in the colonization of water systems by *Legionella*. This study was conducted among various regions in the states of Florida and Georgia. Sites in West Central Florida and East Atlanta were above the OSHA action level 2, for levels above 1,000 CFU/mL. Although detection seemed to be clustered in West Central Florida, it is not believed that the geographic region determines how likely *Legionella*is to grow in cooling towers (Dooling et al., 2015; Prussin, Schwake, & Marr, 2017). Although geographic location is not a predictive factor, climate may play a role in the success of *Legionella* growth in cooling towers (Prussin, Schwake, & Marr, 2017). *Legionella* follows waterborne pathogen trends by having a peak incidence of outbreaks in summer (Phan et al., 2014). In our study a trend was observed in West Central Florida, but other factors are more likely to produce those results, such as the age of the cooling towers, which has been found to be a predictive factor for the colonization of *Legionella* (Brundrett, 2003; Luksamijarulkul et al., 2014). The cooling towers located in the West Central Florida were constructed sometime between the 1960s and 1970s. In contrast, the remaining cooling tower sites located in East Atlanta, West Atlanta, Central Florida, and Northeast Florida are believed to have been constructed sometime in the 1990s. As a cooling tower evaporative condenser gets older, debris and biofilm may accumulate in areas where they cannot be reached. The debris has nutrients necessary for *Legionella* replication, and the biofilm provides a shield against the biocide's bacterial cell penetration (Brundrett, 2003; Hoffman, Friedman, & Bendinelli, 2008).

The industrial facilities where the cooling tower sites are located differ in use. Cooling tower sites CTS-1, CTS-2, CTS-3, CTS-4, CTS-5, CTS-6, and CTS-8 facilities' primary use is for food manufacturing and packaging. As for cooling tower sites CTS-7, CTS-9, CTS-10, and CTS-11, their locations are warehouse-type facilities with no food production or packaging activities. It seems that the use of the facility where cooling towers are located plays a role in the success of *Legionella* colonization. The cooling towers are located on the roofs of all the facilities surveyed. It seems that food production creates airborne food particles that are constantly in contact with the cooling towers' environment. As part of the HVAC system, the evaporative condensers receive air circulated from the system and from inside the facility containing food particles. Also, the towers' location on the roof puts them adjacent to furnace exhaust and air plenum exhaust, in which food particles can become airborne into the environment. With air movement, these particles are likely to travel and are probably being caught by the cooling tower system, providing another nutrient source for *Legionella* and thus encouraging its replication. Interestingly, *Legionella* seem to be more like detected at facilities where food production and packaging activities were performed, and less likely at warehouse-only facilities. Comparisons of *Legionella* isolation have been made, but normally between hospitals, offices, fountains, and cooling towers—where cooling towers presented the highest incidence of *Legionella* isolation (Kim et al, 2014)—but not for food manufacturing–specific sites.

Comparison with Previous Studies

In a similar study to this one conducted by Li et al. published in 2015, "Prevalence and Molecular Characteristic of Waterborne Pathogen Legionella in Industrial Cooling Tower Environments," it was found that 35.7% of the 255 industrial cooling tower water samples tested positive for the presence of *Legionella*. One of the strengths of the study done by Li was the large sample size, with water samples collected in 22 factories in China. In this study the investigators collected samples every month during a 12-month period. One of the main differences between the study performed by Li et al. is that the latter group collected samples right before the biocide was applied to the cooling towers. In our study, we did not discriminate by biocide application, since in the facilities tested in our study, the biocide is applied constantly by an engineered injector to the water line.

A difference with the Li et al. study is that of the sample collection volume; their study collected 500 mL, while ours collected 1,000 mL.

Although Li et al. described the facilities as industrial, their study did not specify what type of activities were conducted. Therefore, the likelihood of *Legionella* growth due to industrial activities could not be examined.

Study Limitations

A limitation of this study is that *Legionella* levels could have been under or overestimated during both sampling events, due to the bacterium's ability to shield itself inside amoeba. Another limitation of this study was that the information regarding the management plan implementation relied on records from the management of the facilities. Due to privacy issues, the records of the maintenance management plan were not provided to the investigator of this study. The final limitation is that only two samples were collected from the same evaporative condenser. The sample collected during 2016 served as the baseline and the 2017 sample served as the follow up sample.

Conclusions

This research study measured the Legionella counts in cooling towers before and after the constant biocide application and implementation of a maintenance management plan. Responses to the research questions are as follow:

1- Does routine biocide application improve *Legionella* control in cooling towers based on water monitoring performed within a one-year span? Based on the data obtained for the year monitored, revealed that applying biocide will help reduce *Legionella* levels within cooling towers. Furthermore, the biocide selection will also play a role in the outcomes of the water treatment for *Legionella* control

2- Does adherence to a Legionella maintenance management program improve Legionella control in cooling towers? From this study having a maintenance management plan will help reduce the *Legionella* growth in cooling towers. Based on the outcomes obtained in this study implementation of scheduled maintenance, recordkeeping, and training of the individuals involved in the cooling tower operation resulted in lower levels of *Legionella*.

3- Does cooling tower sites' geographic location influence the outcome of routine biocide application and adherence to a management program? The data presented in this study showed no differences between the geographic locations of the cooling towers sites and the detection of *Legionella*, that said it is concluded that geographic location does not play a role in the likelihood of *Legionella* growth in cooling towers.

4- Does the intended use of a cooling tower site's facility have an effect on Legionella counts? Based on the data presented in this study the intended use of the facility where the cooling towers where located did showed a predisposition to *Legionella* growth. Those cooling tower sites primarily devoted to food production showed a tendency of having *Legionella* detection. This observation suggests that a facility's use influences the possibility of isolating *Legionella* from cooling towers.

Future Research

Future, research involving cooling tower sampling in industrial sites should include air samples for bacteria to discover whether their presence in the air correlates with their presence in the water. Another future research study should include water sampling in industrial sites where food is produced or packaged to check for *Legionella* and identify if the process held in the facility influence the growth of the bacterium and should be carried over a longer period of time.

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APPENDIX A

IRB Determination Letter

UNIVERSITY OF SOUTH FLORIDA

RESEARCH INTEGRITY AND COMPLIANCE
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10/6/2017

Adelmarie Bones
Environmental and Occupational Health
Tampa, FL 33619

RE: **Not Human Subjects Research Determination**

IRB#: Pro00032711

Title: Biocide substitution and management plan implementation effectiveness for the control of Legionella pneumophila in cooling towers water.

Dear Ms. Bones:

The Institutional Review Board (IRB) has reviewed your application. The activities presented in the application involve methods of program evaluation, quality improvement, and/or needs analysis. While potentially informative to others outside of the university community, study results would not appear to contribute to generalizable knowledge. As such, the activities do not meet the definition of human subject research under USF IRB policy, and USF IRB approval and oversight are therefore not required.

While not requiring USF IRB approval and oversight, your study activities should be conducted in a manner that is consistent with the ethical principles of your profession. If the scope of your project changes in the future, please contact the IRB for further guidance.

If you will be obtaining consent to conduct your study activities, please remove any references to "research" and do not include the assigned Protocol Number or USF IRB contact information.

If your study activities involve collection or use of health information, please note that there may be requirements under the HIPAA Privacy Rule that apply. For further information, please contact a HIPAA Program administrator at (813) 974-5638.

Sincerely,

VJorgensen MD

E. Verena Jorgensen, M.D., Chairperson
USF Institutional Review Board