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Bioaerosols Associated with Evaporative Cooler Use in Low-Income Homes in Semi-Arid Climates

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ABSTRACT

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Asthma is the leading chronic illness in children in the United States. Since children in the U.S. spend a majority of their time indoors there is an increased need to understand key sources of daily asthma triggers in the home. Bacterial endotoxin, dust mite allergens and β-D-glucan have been shown to be potent inducers of asthma attacks, and high levels of these allergens in homes can trigger attacks in those with asthma. We aim to better understand the risks to those with asthma that might be associated with evaporative cooler (EC) use in low-income homes. ECs are often promoted because of their low energy consumption and decreased environmental impact compared to central air conditioning (AC). Because of their lower cost, ECs are more widely used in low-income homes. ECs use evaporation to cool the air, which leads to higher indoor relative humidity. This may create an ecological niche for house dust mites in semi-arid climates where they are normally absent. EC sump water also provides an ideal environment for bacteria and fungi to grow, possibly resulting in EC loading the air with more potential asthma triggers than central air conditioning. We sampled low-income homes around Utah county with central air and evaporative cooling and tested them for the presence of dust mite allergens, β-D-glucan and endotoxin. There were significantly higher levels of endotoxins and β-(1→3)-D-glucans in the EC homes compared to the AC homes, with increased odds of dust mite allergen prevalence but not at clinically significant levels. These findings suggest that in semi-arid environments, endotoxin and β-(1→3)-D-glucan levels in homes with evaporative coolers are more elevated than dust mite allergens.

Keywords: asthma, hygiene hypothesis, endotoxin, β-(1→3)-D-glucan, Der p 1, Der f 1

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CHAPTER 1: Asthma and Bioaerosol Exposure Through Evaporative Coolers

The content of this chapter is being prepared to be submitted for publication as a review on asthma and allergen exposure due to evaporative coolers.

1.1 Introduction

In recent years, a dramatic increase in asthma and allergy prevalence in children, especially in developed countries, has led to increased interest in determining the causative agents and better prevention methods [1]. Asthma is a chronic condition of the lungs that causes tightening of the muscles surrounding the airways. Airways can become swollen, inflamed and clogged with mucus making it difficult to breath [2]. In the United States asthma is currently the third leading cause of hospitalization for children age 0-15 years [3]. Asthma can negatively affect many aspects of children's lives, including mental health and school attendance [4].

The exact underlying cause of asthma continues to be elusive, but it is generally accepted to be due to a combination of genetic and environmental factors [2]. Studies looking at familial incidence of asthma have identified mutations in the *HLA* and *ADAM33* genes that result in higher risk for development of asthma [5]. In an asthma attack, epithelial cells and cells of the immune system such as dendritic cells recognize foreign antigen and trigger the production of cytokines, interleukins and bronchoconstrictors. This leads to muscle contraction, inflammation and closure of airways [6,7]. Inflammation in the lungs is caused by invasion of eosinophils and type 2 helper T cells and subsequent cytokine production as well as immunoglobulin E (IgE) antibody production [8]. When an individual is sensitized, a number of different substances may be recognized as foreign elements by the immune system (including pet dander, dust mite allergens,

endotoxin, fungus, and secondhand smoke) and can trigger asthma attacks [9]. Particulate matter in an urban environment has also been shown to be an important asthma trigger [10-12]. Children in the U.S. spend an average of 70% of their time indoors at home, resulting in an increasing need to understand factors in the home that can contribute to and induce asthma attacks [13].

Evaporative coolers (EC), also known as swamp coolers, are an energy efficient form of air conditioning typically used in dry climates. Over 4 million ECs were in use in the U.S. in 1999, with a heavy concentration of usage in the Rocky Mountain States [14]. Today approximately 29% of homes in the Rocky Mountain States use ECs [15]. Due to the nature of how evaporative cooling works, its use has potential to significantly influence the levels of asthma triggers in the home such as dust mite allergens, endotoxin derived from bacterial cell walls and β-D-glucan, an important component of fungal cell walls, thus creating an atmosphere conducive to asthma attacks.

1.2 Evaporative vs. central cooling mechanisms

ECs pull hot, dry air from outside across a wet evaporative pad that is constantly moistened by water from a water distributor. As the air passes through the evaporative pad the water evaporates, transferring the heat from the outdoor air into latent heat stored in the form of water vapor. The water vapor is blown into the house with the cooled air and the liquid water is then drained into the water reservoir where it can circulate back through the system [16]. A fan then blows the cool air into the home (Figure 1-1). As a result of evaporation, ECs lower indoor air temperature while simultaneously increasing humidity and making the home more hospitable for certain types of bacteria, fungus and dust mites [17]. A study in the Great Basin region (NV) found that children living in EC homes were significantly more likely to be sensitized to dust mite allergens than individuals living in non-EC homes [18]. Conversely, another study in Colorado

showed no heightened dust mite or mold sensitization due to EC use [19]. Research done in Utah County, Utah showed higher levels of endotoxin and β-glucan as well as increased odds of dust mite allergen presence due to EC use [20,21]. The health implications of EC usage in the Rocky Mountain region remain unclear, but it is clear that EC use significantly influences levels of bioaerosols in the home, while central cooling provides a cleaner alternative by condensing water vapor onto the air conditioner's evaporator coils, in turn lowering indoor relative humidity [22].

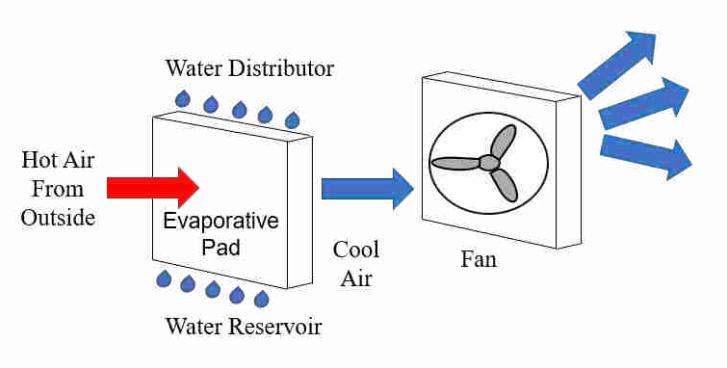

Figure 1-1. The principles behind evaporative cooling. Hot air is pulled through a moist evaporative pad where it is cooled and then distributed throughout the home.

1.3 Early life antigen exposure and the hygiene hypothesis

Whether or not this antigen exposure due to air conditioning type can be helpful or harmful is a complicated issue and remains unresolved. There are more than 1,600 genes involved in innate and adaptive immune responses, yet at birth the immune system is relatively immature and must develop over the course of one's life [23]. Early post-natal life exposure is essential for immune system development and the microbiota individuals are exposed to plays a key role in the education and development of the immune system [23,24]. The hygiene hypothesis suggests that an environment with high incidence of infectious diseases protects against allergy development and certain autoimmune diseases. Epidemiological evidence seems to support that claim, showing that children with older siblings, early daycare attendance and exposure to

livestock have a lower incidence of allergic disease [25]. Children living in urban environments have also been shown to be protected against asthma development if their indoor home environment closely models the indoor microbiota found in farm homes [26]. The hygiene hypothesis mainly focuses on microbial exposures from the environment, food and drink and domestic animals. Recent evidence, summarized in the biodiversity hypothesis, suggest that it should extend to living environments in general, proposing that a decrease in microbiological biodiversity is related to an increase in inflammatory disease [27]. The hygiene hypothesis has been linked with immune regulatory pathways and regulatory T cells as well as the helper T cell response [1]. Specifically, farm exposure during pregnancy is associated with an increase in number and function of regulatory T cells within cord blood as well as a reduction in T helper cell type 2 cytokine production [28].

1.4 Asthma and T helper cell 2 (TH2) response

The lungs comprise a large surface area constantly exposed to the external environment and airborne antigens. Many of these antigens do not pose a threat to the body. As a result the immune system has several control mechanisms in place to regulate unnecessary responses to allergens and prevent potential subsequent inflammation and damage to lung epithelial cells [1]. Early exposure to allergens such as pet dander and endotoxin has been shown to prevent allergic disease and asthma from developing by encouraging T helper cell 1 (TH1) type development over a TH2 response [29]. TH1 cells produce and secrete interferon-gamma, interleukin (IL)-2, and tumour necrosis factor (TNF)-beta. These cytokines help activate macrophages and are responsible for cell-mediated immunity and phagocyte-dependent protective responses. TH2 cells produce interleukin (IL)-4, IL-5, IL-10, and IL-13. These cytokines are responsible for strong antibody production, eosinophil activation, and inhibition of several macrophage

functions, thus providing phagocyte-independent protective responses [30]. Due to the differences in cytokine production and subsequent antibody production and cell activation, TH2 cells can promote a spectrum of diseases including asthma and allergic disorders. Approximately 3 billion people worldwide are afflicted with diseases resulting from TH2 responses, with 100 million new cases of asthma expected in the United States alone by the year 2025 [31,32]. IL-4 and IL-13 production are of particular concern with TH2 responses. They drive most of the key characteristics associated with TH2 responses including immunoglobulin E (IgE) production, an important antibody in allergy and inflammatory responses, smooth muscle contraction and mucus production, which are all symptoms of asthma attacks [33,34]. Exposure to various elements in house dust has been found to influence either TH1 or TH2 driven responses [29,35]. Certain elements derived from common house dust mites have been shown to drive TH2 responses [35], while early endotoxin exposure has been shown to encourage TH1 responses [29]. Dust mite allergens and endotoxin settle in house dust and are then resuspended and breathed in with cleaning or as children play. Several factors have potential to influence allergen levels in settled house dust including presence of pets, pests (e.g. cockroaches, rats) as well as location of the home (e.g. residential or rural) [36]. Because of the way evaporative coolers work they have significant potential to introduce more levels of allergens into the home that can drive these T helper cell responses.

1.5 Evaporative cooling as a distributor of endotoxin

Endotoxin, also known as lipopolysaccharide or LPS, is a soluble component on the cell wall of gram-negative bacteria [37]. It is composed of a hydrophobic lipid section (lipid A), a hydrophilic core polysaccharide chain and a repeating hydrophilic O-antigenic oligosaccharide side chain [38]. Endotoxin is a pathogen associated molecular pattern (PAMP) that can be bound

and recognized as foreign by pathogen recognition receptors (PRRs) located on various cells of the immune system [39]. High levels of endotoxin in the body can lead to inflammation and vasodilation when recognized by transmembrane protein toll-like receptor 4 (TLR4) [40,41]. TLR4 is a PRR expressed on a wide variety of cell types, including dendritic cells, macrophages, neutrophils and B cells. Endotoxin binding of TLR4 activates transcription factor NF-κB, and the subsequent production of pro-inflammatory cytokines [41](Figure 1-2).

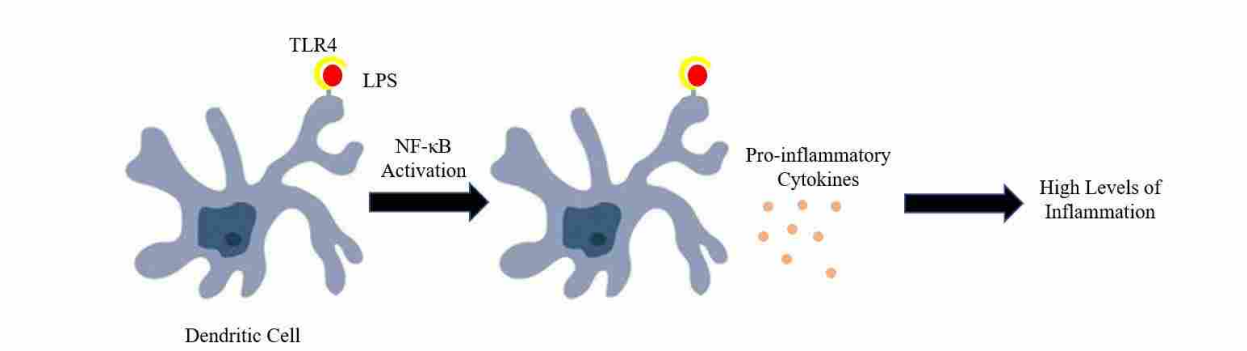

Figure 1-2. The immune response to LPS. TLR4 recognition of LPS (endotoxin) leads to activation of NF-κB and production of pro-inflammatory cytokines, resulting in an inflammatory response.

Development of asthma and allergic disease are attributed to a combination of genetic and environmental factors. Research has shown that two missense mutations located in the extracellular domain of the TLR4 receptor are associated with differences in reaction to inhaled endotoxin. Heterozygous individuals demonstrated a hypo-responsive phenotype to endotoxin [37,42]. Early childhood endotoxin exposure has been shown to be protective against the development of asthma. This can be observed in the Amish and Hutterite communities. Both populations share common ancestry, but asthma is 4 to 6 times lower in Amish children, while Amish dust endotoxin levels are 6.8 times as high. Mice exposed to this Amish dust were protected from development of asthma, while mice exposed to Hutterite dust were not [43]. Similarly, a study was done with children in the Alps area, consistently showing an inverse

relationship between endotoxin exposure and the prevalence of asthma [44]. Recent research published in *Nature* took this idea one step further, showing that exposure to all bacteria, not just gram-negative, as well as fungal exposure protects from asthma development [26]. However, exposure to endotoxin in the home is also known to obstruct airflow in adults and children that are already asthmatic [45,46]. Studies have shown housing variables such as living on a farm [47], having more residents in the home and presence of cockroaches [48,49] lead to higher levels of endotoxin in the home, but little is known about the relationship between EC use and house dust endotoxin levels. Evaporative cooling creates a warm, moist environment inside the unit that is conducive to the growth of gram-negative bacteria, potentially leading to higher levels of endotoxin in the home. Evaporative cooler sump water has tested positive for high levels of gram-negative bacteria. Since this water is used to cool the hot air pulled from outside, evaporative cooling could be a source of endotoxin distribution in low-income homes more than central cooling [50]. Whether this type of endotoxin exposure is helpful or harmful for those living in the homes cooled by an evaporative cooler remains unclear.

1.6 Evaporative cooling as a distributor of β-(1→3)-D-glucan

The warm, moist environment created by ECs could also potentially lead to an increase in β-D-glucan levels in the home. Mold, fungus and dampness have been shown to exacerbate asthma symptoms and trigger attacks. Since β-D-glucan is a major component in fungal cell walls, measuring levels of β-D-glucan can lead to a better understanding of fungal exposure from ECs in the home [51].

β-(1→3)-D-glucan is a polysaccharide located in the fungal cell wall. B-glucans can trigger various immune responses including activation of neutrophils, macrophages, complement and possibly eosinophils [52-56]. B-glucans are of particular interest for their immunomodulating

properties. They have been shown to enhance cytotoxic activities of macrophages [57], and functional maturation of dendritic cells [58] as well as to prevent cancer promotion and progression [59]. While exact mechanisms of how β-(1→3)-D-glucan exposure might trigger asthma remain elusive, a few positive associations with asthma and β-(1→3)-D-glucan have been discovered. A study looking at children from infancy to 7 years of age showed that children exposed to higher environmental relative moldiness index (ERMI) at the age of 1 year were more likely to develop asthma at 7 years of age [60]. Another study used a mouse model to further understand what exactly happens in β-glucan exposure. Mice exposed to inhaled β-glucan showed a slightly enhanced TH2 response while mice exposed to inhaled β-glucan in conjunction with house dust mites showed a significantly enhanced TH2 response. Various other studies also suggest there is a link between fungal exposure and asthma [61,62], however other evidence shows that perhaps β-(1→3)-D-glucan is not actually the causitive agent, with health effects actually being caused by inhaled endotoxin [51,63]. A study done in mice looking at ingestion of WGP®, a preparation of β-1,3/1,6-glucans, showed that β-glucan ingestion attenuates the TH2 response important in asthma attacks. Orally administered β-glucan in mice was shown to significantly reduce pulmonary eosinophils and TH2 cytokine production but had no effect on IgE levels. The reduction seen did not reach a threshold to see histological effects, nor did it have an impact on lung pathology, indicating that β-glucans could potentially mitigate asthma symptoms, but is best seen as an option in conjunction with other treatments[8]. Another study showed β-glucan administration to subjects with seasonal allergic rhinitis also demonstrated a reduction in IL-4 and IL-5 levels [64]. Another study still showed β-glucan administration caused a reduction in asthmatic symptoms [65]. Despite no concrete evidence of β-(1→3)-D-glucan being the causitive agent of asthma attacks, many studies show that exposure to inhaled β-(1→3)-D-glucan increases asthma severity [66,67].

Research has shown that immune responses induced at mucosal surfaces create much more tolerogenic dendritic cells and certain types of regulatory T cells because of a need to discriminate against commensal flora and invading pathogens [68,69]. The seemingly contradictory reactions to ingested and inhaled β-glucans are consistent with what is seen in mucosal and respiratory immune responses.

Research in the great basin desert region showed children living in homes cooled with ECs are more likely to be sensitized to fungal exposure [70]. Homes with ECs present with significantly different fungal populations compared to air conditioned homes, which could potentially explain this difference in fungal sensitization in children [70,71]. ECs require significant maintenance to prevent mold and fungal growth, with a frequent changeout of the cooling pad required to avoid this growth. It is recommended that they be changed out twice during the cooling season, even though cooling pads are designed to last three to five years, because of this people normally don't replace cooling pads as often as recommended [72]. This potential for fungal growth within the system and the more diverse fungal populations in EC homes could likely mean risk for exposure to higher levels of β-glucan in the home.

1.7 Evaporative cooling creates a potential ecological niche for dust mites

The increase in humidity associated with evaporative cooling could create an ecological niche for house dust mites that otherwise would not be able to thrive in a semi-arid climate. To survive, house dust mites primarily acquire water from humid air [73,74]. Relative humidity (RH) levels necessary for the survival of house dust mites range from 55.0% to 75.0% at a temperature of 15.0° C to 35.0° C [74,75]. Evaporative cooling has been shown to increase RH to linger either just below or barely within the range needed for dust mite survival in semi-arid climates, whereas central cooling RH lingers around 40%, just outside that range [76].

An increase in dust mite numbers means an increase in dust mite allergens. Der p 1 and Der f 1 are two common dust mite allergens known to trigger asthma attacks [77]. Der p 1 originates from *D. pteronyssinus* and Der f 1 from *D. farinae*, two common house dust mites [20]. These antigens are derived from enzymes in the gut of these two dust mite species. The antigens are expelled in dust mite feces. The feces can become suspended in the air with dust and an asthmatic individual can breathe that in, potentially initiating an asthma attack. Dust mite allergens are also a leading cause of atopic sensitization and allergy. Exposure to allergens from *Dermatophagoides pteronyssinus* and *D. farina* especially has been shown to induce allergic sensitization [78]. For this sensitization to happen, dust mite allergens must be present at clinically significant levels. This threshold has been determined to be 2 µg of dust mite allergens per gram of dust [79]. Some studies have shown a correlation between EC use and prevalence of these dust mite allergens in homes [17,80] while others have shown no correlation between EC use and dust mite allergen presence in the home [76,81]. Studies done in Colorado and other locations with climates similar to that of Utah showed a link between EC use and higher levels of dust mite allergens Der p 1 and Der f 1. A similar study was performed in Utah county, Utah in middle-income homes but found no link between EC use and high levels of dust mite allergens. A brief pilot study performed in Utah comparing dust mite allergen levels in low-income homes with evaporative coolers with the previous data in middle-income homes showed a drastic increase in the number of homes testing positive for dust mite allergen levels [20,76], suggesting that socioeconomic status may potentially explain these seemingly contradictory findings.

1.8 Endotoxin and dust mite allergen exposure as a result of socioeconomic status

A previous study showed that middle-income homes in Utah county had consistent levels of dust mite allergens Der p 1 and Der f 1 regardless of air conditioning type [76], with similar

findings in other studies [81,82]. However, in a pilot study, the prevalence of the same dust mite allergens in the same Utah community greatly increased in low-income homes using evaporative cooling [20], suggesting that perhaps low-income homes using evaporative cooling could have higher levels of dust mite allergens and other asthma triggers such as endotoxin than low-income homes that use central cooling. 68.2% of low-income homes tested positive for either Der p 1 or Der f 1, whereas only 25% of middle-income homes in the same community tested positive for these allergens [20]. Since this pilot study did not contain a comparison group of low-income homes with central cooling, a larger scale study was performed to determine if low-income homes with central cooling and evaporative cooling have different levels of allergens present.

In the larger scale study, dust samples collected from low-income homes using evaporative coolers in Utah county were 2.29 times more likely to test positive for dust mite allergens Der p 1 and Der f 1 than low-income homes with central air, however these values were not above the 2 μg of dust mite allergen per gram of dust threshold needed for allergic sensitization (unpublished data presented in chapter 2 of this thesis). This could potentially mean that even though homes with evaporative coolers result in an increase in dust mite allergen exposure, this is not clinically significant for allergy and asthma development. However, the homes using ECs also had significantly higher levels of endotoxin and β-glucan than those with central air (unpublished data presented in chapter 2 of this thesis). This could potentially suggest that another factor besides just air conditioning type, such as socioeconomic status, is also influencing levels of allergens.

1.9 Evaporative cooler benefits and usage

EC use is often promoted in dry regions for its energy efficiency; however, government programs tend to overlook the potential health concerns presented by ECs for people with

asthma. Since the 1950s, ECs have been a popular, low-cost alternative to AC in dry climates, particularly when energy costs are high [14,83]. Current concerns about energy consumption and climate change are driving a resurgence in ECs, and rebate programs focus heavily on the 50 – 80% energy savings compared with AC. Currently, energy companies in California, Idaho, Utah, Wyoming, New Mexico, and Colorado offer rebates ranging from $150 to $700 for purchase of a qualified EC. However, rebate programs do not generally mention health concerns for individuals with asthma.

1.10 Evaporative cooler use and health effects in relation to asthma

A recent study on middle-income homes in Utah county, Utah found that endotoxin levels were three to six times higher in EC homes compared with AC homes [21]. Similar findings were reported in a recent study conducted in the Reno, Nevada area [71]. There is growing evidence that early life exposure to endotoxins may be protective against the development of asthma and other allergic diseases later in life [29,84]. However, in asthmatic adults and children, inhaled endotoxin is a known asthma trigger [45,46,85]. Wet EC pads also appear to change the microbial diversity within homes. Recent research found significantly higher levels of hydrophilic fungi in air samples from EC vs. non-EC homes, many of which were highly allergenic [71]. This study also reported significant diversity in fungal species between EC and non-EC homes, with EC homes being more likely to harbor species associated with allergic sensitization. A recent study in Denver, Colorado showed that evaporative coolers were not responsible for dust mite or mold sensitization [19] however, the three to six-fold higher endotoxin levels and four-fold higher fungi levels found in EC homes may significantly contribute to asthma exacerbation in sensitized individuals. However, to date, there are no interventions

reported in the literature to reduce bioaerosol exposures in EC homes, warranting future studies in this area.

1.11 Summary of research chapters

Chapter 2 contains a research paper that has been submitted for publication to a scientific journal and is currently under review. We collected samples from low-income homes in Utah County, Utah with either evaporative cooling or central air and tested them for presence of three bioaerosols: dust mite allergens, endotoxin and β-(1→3)-D-glucan. Our findings show that low-income homes in Utah County that use evaporative coolers have higher levels of all three allergens in the home, which could have health implications for asthmatic individuals.

Chapter 3 contains a summary of the main findings on allergen exposure and evaporative coolers in low-income homes. We also propose future research plans to further understand what this allergen exposure could mean for asthmatic individuals as well as characterization of fungal species present in homes with evaporative cooling and central air.

CHAPTER 2: Associations Between Evaporative Cooling and Dust Mite Allergens, Endotoxins, and β-(1→3)-D-Glucans in House Dust: A Study of Low-Income Homes

The content of this chapter has been submitted to a scientific journal for publication and is currently under review.

Abstract

Recent work suggests that evaporative coolers increase the level and diversity of bioaerosols, but this association remains understudied in low-income homes. We conducted a cross-sectional study of metropolitan, low-income homes in Utah with evaporative coolers (n = 20) and central air conditioners (n=28). Dust samples (n=147) were collected from four locations in each home, and analyzed for dust mite allergens Der p 1 and Der f 1, endotoxins, and β-(1→3)-D-glucans. In all sample locations combined, Der p 1 or Der f 1 was significantly higher in evaporative cooler versus central air conditioning homes (OR = 2.29, 95% CI = 1.05 – 4.98). Endotoxin concentration was significantly higher in evaporative cooler versus central air conditioning homes in furniture (geometric mean (GM) = 8.05 vs. 2.85 EU/mg, $P < 0.001$) and all samples combined (GM = 3.60 vs. 1.29 EU/mg, $P = 0.031$). β-(1→3)-D-glucan concentration and surface loads were significantly higher in evaporative cooler versus central air conditioning homes in all four sample locations and all samples combined ($P < 0.001$). Evaporative coolers are often incentivized for energy savings in dry climates, but there is little data on potential health risks or benefits of their use, warranting a longitudinal health study.

2.1 Introduction

Residential exposure to bioaerosols (i.e. inhalable particles of microbial, plant, or animal origin) appears to be responsible, paradoxically, for both protection against and initiation of serious allergic diseases. Early-life bioaerosol exposures play a key role in shifting the immune system either toward or away from a T helper cell 2 (TH2) imbalance and associated atopy [86,87]. Conversely, exposure to residential bioaerosols can lead to sensitization with multiple allergy-type symptoms in asthmatic and non-asthmatic individuals [88]. Children spend approximately 60 – 80% of their time [89], and adults spend approximately 70% of their time indoors at home [90,91], making the home a key microenvironment for bioaerosol exposure. The bioaerosol profile of an individual home varies widely based on multiple housing factors, including geographical location [48,92-96], presence of indoor or outdoor animals [48,49,92-99], living on a farm [92,97], number of occupants [48,49,93-95], cleanliness and presence of cockroaches [48,98], presence of carpeting or rugs [94,97], and other factors. A few studies also suggest that evaporative coolers may significantly alter the bioaerosol profile in homes for immunologically important exposures such as house dust mite (HDM) allergens Der p 1 and Der f 1, bacterial endotoxins, and β-(1→3)-D-glucans [17,21,71,80].

Evaporative coolers are predominantly used in climates with low relative humidity (RH) and seasonally high outdoor temperatures [83] such as the Rocky Mountain States in the U.S. Residential evaporative coolers remove heat from dry ambient air by drawing the air across wetted filter media, resulting in lower indoor air temperature, but higher RH [17,76]. The higher RH from evaporative coolers may create an ecological niche for HDMs to survive in arid and semi-arid climates [17,80], potentially exposing home occupants to mite allergens [18]. Furthermore, untreated evaporative cooler sump water provides a growth medium for Gram-negative bacteria

during summer months [18], and may be a significant source of residential endotoxin load in homes [21,71]. Early-life exposure to endotoxins, the lipopolysaccharide portion of the cell wall in Gram-negative bacteria, may confer protection against the development of allergic diseases, including asthma [18,43,44,84,100] , while simultaneously causing airway inflammation and asthma symptoms in children and adults [45,46,51,85]. Finally, fungi are more common, and fungal species associated with allergies are more prevalent, in homes with evaporative coolers compared to homes with other forms of air conditioning [71,101]. β-(1→3)-D-glucans, polysaccharides found in the cell wall of fungi, plants, and some bacteria, are important immunologically because they are associated with airway inflammation and decreased lung function [102-106]. We hypothesized that evaporative coolers may also be associated with higher levels of mold-related bioaerosols such as β-(1→3)-D-glucans, although we are not aware of any prior studies reporting this.

Although a growing body of research shows that evaporative coolers are associated with higher levels and varying types of immunologically important bioaerosols in homes, most prior studies on this topic do not report socioeconomic factors related to their study homes. Lower socioeconomic status is associated with increased asthma incidence and morbidity in affluent countries [107-109], and factors such as household income and occupant density may be important modifying factors for indoor bioaerosol loads. The purpose of this study, therefore, was to compare bioaerosol levels specifically in low-income households based on use of evaporative coolers or central air conditioners.

2.2 Methods

2.2.1 Study design

We conducted a cross-sectional study which included homes (N=48) that were identified by recruiting individuals exiting the Woman, Infants, and Children (WIC) office at the Utah

County Health Department (UCHD). Research assistants were stationed at a booth outside of the WIC office, where they contacted potential participants in English and Spanish as they exited the office. Participants in the study were given study flyers in either English or Spanish, and a screening questionnaire to determine if their home met criteria for the study. Exclusion criteria for the study were (1) use of humidifiers or vaporizers, (2) previous water damage covering more than 9.29 m^2 (100 ft^2), (3) home newer than 5 years old, and (4) household income ≥ 100% of the 2017 Federal Poverty Guidelines. All participating homes were in Utah County, Utah and used evaporative cooling (N=20), central air conditioning (N=24), or no air conditioning (N=4). Home visits were conducted between July and October 2017. At each home visit, study personnel collected dust samples and measured indoor temperature and relative humidity (RH). Brigham Young University's Institutional Review Board approved this study (IRB #X17261).

2.2.2 Reservoir dust collection

Dust was collected from four different areas in each home. These areas included the primary adult resident's mattress, the floor in the same room as the mattress, upholstered furniture in the living room, and the living room floor. All bedding was removed from the mattress before sampling to allow a direct sample from the mattress to be taken. A 1 m^2 area of the surface to be sampled was measured and then vacuumed continuously for 3 minutes. Each sample was taken using a Eureka (Bloomington, IL, USA) "Mighty Mite" vacuum (model 3684F) attached to a Duststream® dust collector extension and 40 μm nylon mesh filter (Indoor Biotechnologies, Charlottesville, VA, USA). Prior to sample collection, all research assistants were trained on sampling procedures. Following sample collection, the dust was sieved using a #50 wire mesh (300μm) and then moved to a 15 ml polypropylene conical tube. Samples were

then stored at approximately -20° C until they were analyzed for dust mite allergens Der p 1 and Der f 1, endotoxins, and β-(1→3)-D-glucans.

2.2.3 Der p 1 and Der f 1 extraction and analysis

Der p 1 (*Dermatophagoides pteronyssinus*) and Der f 1 (*D. farinae*) allergens were extracted by suspending 100 mg of dust in 2 mL of phosphate-buffered saline with 0.05% Tween-20 (PBS-T). Samples were agitated for 2 hours at room temperature and then centrifuged for 20 minutes at 2,500 RPM and 4°C. Allergen levels were detected using a two-site monoclonal antibody-based enzyme-linked immunosorbent assay (ELISA) kit (Indoor Biotechnologies, Charlottesville, VA, USA). Sample extracts (100 µL) were added in triplicate to each plate, and a 10-point curve was made using sequential 2-fold dilutions of a known standard in PBS-T with 1% BSA. Samples were read with an optical density plate reader at wavelength 405 nm. Prism 8 software (GraphPad Software, La Jolla, CA, USA) was used to extrapolate allergen concentrations from the standard curve for each plate. The limit of detection (LOD) for both Der p 1 and Der f 1 assays was 0.04 µg/g of dust.

2.2.4 Endotoxin and β-(1→3)-D-glucan extraction and analysis

Extraction of endotoxin and β-(1→3)-D-glucan from dust samples was performed by suspending 100 mg of dust in 2 mL of sterile, pyrogen-free LAL water containing 0.05% Tween-20 (LAL-T), followed by agitation at room temperature for 1 hour. After agitation, samples were centrifuged at 2500 RPM at 4°C for 20 minutes and supernatants were collected and stored at −20°C. For endotoxin testing, sample extracts were diluted in LAL endotoxin-free water and were added in triplicate to each plate. A seven-point curve was made using four sequential 1:10 dilutions of a known standard in LAL endotoxin-free water. Endotoxin levels were measured using a Kinetic-QCL™ assay (Lonza, Walkersville, MD, USA). Samples were read with an

optical density plate reader at wavelength 405 nm. Plate reader software was set up to measure time of onset at 0.200 O.D. units. Microsoft Excel (Microsoft Corporation, Redmond, WA, USA) was used to extrapolate endotoxin concentrations from the onset times for each standard. The LOD for endotoxin was 0.0001 EU/mg.

For β-(1→3)-D-glucan testing, sample extracts were heat treated at 100°C for one hour and diluted in pyrogen-free water. Samples were added in triplicate to each plate. A five-point standard curve was made using sequential 2-fold dilutions. β-(1→3)-D-glucan levels were measured using (1,3)-β-D-Glucan Detection Glucatell® kit based on the Kinetic Onset Time protocol according to manufacturer's specifications (Associates of Cape Cod Incorporated, East Falmouth, MA, USA). Samples were read with an optical density plate reader at wavelength 405 nm. Plate reader software was set up to measure time of onset at 0.03 O.D. units. Microsoft Excel (Microsoft Corporation, Redmond, WA, USA) was used to extrapolate β-(1→3)-D-glucans concentration from a log-log standard curve. The LOD for β-(1→3)-D-glucan was 0.0000625 μg/mg dust.

2.2.5 Indoor temperature and RH measurement

Indoor air temperature and RH measurements were continuously logged every 5 minutes for approximately 72 hours during the sampling period. Collection was performed using Extech SD500 humidity/temperature dataloggers (Extech Instruments, Corp., Waltham, MA, USA) placed 0.91-1.83 m (3-6 ft) above the floor in a central living area within the home and distanced from cooling/heating vents. NIST-traceable calibration of temperature and humidity sensors was performed prior to data collection, and all instruments were found to be within the manufacturer's tolerances of 0.8°C (1.5°F) and 4% RH. A weather monitoring station located on

the campus of Brigham Young University was used to measure outdoor air temperature and RH measurements during the sampling period.

2.2.6 Housing questionnaire

The research team obtained written informed consent and administered a 40-item housing survey at the beginning of each in-home visit. The survey included items regarding the style, age, and size of the home, number of persons, and age and type of mattresses, flooring, and other furniture.

2.2.7 Statistical techniques

All analyses were conducted using SAS version 9.4 (SAS Institute Inc., Cary, NC). The four homes that had no air conditioning were combined with the 24 homes that had central air conditioning for analyses (hereafter referred to as central air conditioning homes). Geometric means (GM), geometric standard deviations (GSD), minimums, maximums and p-values from t-tests were calculated for continuous characteristics of single-family homes and frequencies, percentages and p-values from χ^2 tests were calculated for categorical characteristics according to air conditioning type (central air conditioning vs. evaporative cooler). Occupant density, age of living room furniture and age of bedroom carpet were right skewed, so these variables were natural logarithm transformed.

Seventy-two-hour mean RH and temperature were normally distributed, so arithmetic means (AM), 95% confidence intervals (CI), minimums, and maximums were calculated. All of the house dust analytes (Der f 1, Der p 1, and combined Der f 1 or Der p 1 concentration; endotoxin concentration and surface load, β-(1→3)-D-glucan concentration and surface load) were right skewed, so these variables were natural logarithm transformed and GMs, GSDs or 95% CI, minimums, and maximums were calculated. Linear regression models were used to

calculate unadjusted AM differences, 95% CI, and p-values that estimated associations between air conditioning type or housing characteristics and 72-hour mean RH or temperature. Unconditional exact or large sample/asymptotic approximate logistic regression models were used to calculate unadjusted exact or large sample/asymptotic approximate odds ratios (OR) and 95% CI that estimated associations between air conditioning type or housing characteristics and the odds of Der f 1, Der p 1 and combined Der f 1 or Der p 1 above detection limits for individual sample locations and all sample locations combined. Tobit regression models were used to calculate unadjusted GM ratios, 95% CI, and p-values that estimated associations between air conditioning type or housing characteristics and endotoxin concentration or surface load for individual sample locations and all sample locations combined. Linear regression models were used to calculate unadjusted GM ratios, 95% CI, and p-values that estimated associations between air conditioning type or housing characteristics and β-(1→3)-D-glucan concentration or surface load for individual sample locations and all sample locations combined.

Stepwise variable selection was used to develop unconditional logistic or linear regression models that included housing characteristics that were associated with 72-hour mean RH and temperature, Der f 1, Der p 1, and combined Der f 1 or Der p 1 concentration, and β-(1→3)-D-glucan concentration and surface load. Entry and exit significance levels were set at $\alpha = 0.15$. SAS does not have an option to conduct stepwise variable selection for Tobit regression models, so all housing characteristics were included in multiple Tobit regression models for endotoxin concentration and surface load and housing characteristics that had p-values less than $\alpha = 0.15$ were identified. Unconditional logistic, Tobit, or linear regression models were then used to calculate associations between air conditioning type and 72-hour mean RH and temperature, Der f 1, Der p 1, and combined Der f 1 or Der p 1 concentration, endotoxin

concentration and surface load, and β-(1→3)-D-glucan concentration and surface load adjusting for housing characteristics that were identified via stepwise variable selection (or multiple Tobit regression models for endotoxin concentration and surface load).

Spearman's rank correlation coefficients were used to estimate unadjusted associations between 72-hour mean RH and temperature, Der f 1, Der p 1, and combined Der f 1 or Der p 1 concentration, endotoxin concentration and surface load, and β-(1→3)-D-glucan concentration and surface load.

2.3 Results

2.3.1 Home characteristics

The geometric mean age of homes was 38.58 years and the geometric mean size was 92.26 m^2 (Table 2-1). The percentages of homes that were apartment, single story, two story, or other styles of homes were 25, 17, 27, and 31, respectively. The arithmetic mean number of residents was 4.79 and the geometric mean occupant density was 4.79 residents per 100 m^2. The geometric mean ages of the bedroom carpet, bedroom mattress, living room carpet, and living room furniture were 94.62, 35.33, 61.47, and 53.29 months, respectively. Home age was the only characteristic that was statistically significantly different (P = 0.002) among homes with central air conditioning (31.70 years) and homes with evaporative coolers (51.53 years). The average daily outdoor temperature and RH during the study period were 19.13°C and 41.03% respectively.

Table 2-1. Characteristics of low-income homes in Utah County, Utah, Summer 2017.

Characteristic	Homes, n	Missing, n	GM	GSD	Min	Max	Dust Samples, n (%)	Missing Dust Samples[†], n	p-value[‡]
Central air conditioning or none									
Total	28						86 (100)	26	
Home age, years	28		31.70	2.13	10.00	137.00			
Home size, m^2	28		96.22	1.50	46.45	241.55			
Style of home, n (%)									
Apartment	7 (25)						23 (27)	5	
Single story	2 (7)						7 (8)	1	
Two story	9 (32)						28 (33)	8	
Other[§]	10 (36)						28 (33)	12	
Residents, n	28		4.71[¶]	1.80[¶]	1.00	8.00			
Occupant density, residents/100 m^2	28		4.50	1.54	0.98	11.66			
Bedroom carpet age, months	26	2	91.62	4.07	1.00	924.00			
Bedroom mattress age, months	28		30.13	3.45	1.00	156.00			
Living room carpet age, months	25	3	67.43	4.37	1.00	960.00			
Living room furniture age, months	28		56.19	2.28	12.00	324.00			
Sample location, n (%)									
Bedroom carpet							26 (30)	2	
Bedroom mattress							16 (19)	12	
Living room carpet							24 (28)	4	
Living room furniture							20 (23)	8	
Evaporative cooler									
Total	20						65 (100)	15	
Home age, years	19	1	51.53	1.43	37.00	112.00			0.002
Home size, m^2	19	1	86.72	1.40	41.81	140.00			0.39
Style of home, n (%)									
Apartment	5 (25)						17 (26)	3	

Characteristic	Homes, n	Missing, n	GM	GSD	Min	Max	Dust Samples, n (%)	Missing Dust Samples[†], n	p-value[‡]
Single story	6 (30)						19 (29)	5	
Two story	4 (20)						15 (23)	1	
Other[§]	5 (25)						14 (22)	6	0.19[††]
Residents, n	20		4.90[¶]	1.92[¶]	2.00	11.00			0.75[‡‡]
Occupant density, residents/100 m^2	19	1	5.26	1.53	2.15	10.09			0.95
Bedroom carpet age, months	17	3	99.38	4.21	2.00	876.00			0.89
Bedroom mattress age, months	20		44.17	2.76	8.00	240.00			0.37
Living room carpet age, months	17	3	53.65	4.40	2.00	876.00			0.96
Living room furniture age, months	20		49.48	2.88	6.00	240.00			0.23
Sample location, n (%)									
Bedroom carpet							20 (31)		
Bedroom mattress							16 (25)	4	
Living room carpet							19 (29)	1	
Living room furniture							10 (15)	10	
All homes									
Total	48						151 (100)	41	
Home age, years	47	1	38.58	1.94	10.00	137.00			
Home size, m^2	47	1	92.26	1.46	41.81	241.55			
Style of home, n (%)									
Apartment	12 (25)						40 (26)	8	
Single story	8 (17)						26 (17)	6	
Two story	13 (27)						43 (28)	9	
Other[§]	15 (31)						42 (28)	18	
Residents, n	48		4.79[¶]	1.83[¶]	1.00	11.00			
Occupant density, residents/100 m^2	47	1	4.79	1.54	0.98	11.66			
Bedroom carpet age, months	43	5	94.62	4.06	1.00	924.00			
Bedroom mattress age, months	48		35.33	3.18	1.00	240.00			

Characteristic	Homes, n	Missing, n	GM	GSD	Min	Max	Dust Samples, n (%)	Missing Dust Samples[†], n	p-value[‡]
Living room carpet age, months	42	6	61.47	4.32	1.00	960.00			
Living room furniture age, months	48		53.29	2.51	6.00	324.00			
Sample location, n (%)									
Bedroom carpet							46 (30)	2	
Bedroom mattress							32 (21)	16	
Living room carpet							43 (28)	5	
Living room furniture							30 (20)	18	

Abbreviations: GM, geometric mean; GSD, geometric standard deviation; Min, minimum; Max, maximum.

[†] Although four dust samples were collected from each house (i.e., 192 total dust samples were collected), dust samples were designated "missing" if not enough dust could be collected to measure the analytes of interest (e.g., because the mattress, furniture, or carpet was newer or made out of leather).

[‡] Estimated via t-tests of the natural logarithm transformed values.

[§] Includes basement apartment (two homes, seven dust samples), condo (one home, three dust samples), duplex (four homes, nine dust samples), four plex (one home, three dust samples), four plex split level (one home, two dust samples), split entry (two homes, five dust samples), trailer (one home, three samples), tri-level split (one home, four samples), and not applicable (two homes, six dust samples).

[¶] Arithmetic mean and arithmetic standard deviation.

[††] Estimated via χ^2 tests.

[‡‡] Estimated via t-tests of the original values

2.3.2 Indoor RH and temperature

Seventy-two-hour RH was not statistically significantly different (P = 0.82) among homes with central air conditioning (AM = 43.91%) and homes with evaporative coolers (AM = 44.54%) (Supplemental Table 2-1). None of the housing characteristics were significantly associated with 72-hour mean RH (Supplemental Table 2-2). Stepwise variable selection for 72-hour mean RH did not select any housing characteristic.

Seventy-two-hour mean temperature was significantly lower (P = 0.008) in homes with evaporative coolers (AM = 23.31°C) compared to homes with central air conditioning (AM = 24.56°C) (Supplemental Table 2-3). None of the housing characteristics were significantly associated with 72-hour mean temperature (Supplemental Table 2-4). Stepwise variable selection for 72-hour mean temperature did not select any housing characteristic.

2.3.3 Der f 1 and Der p 1 allergen concentration

Twenty-two (15%) dust samples were positive (i.e., above detection limits) for Der f 1 (Supplemental Table 2-5) and 16 (11%) dust samples were positive for Der p 1 (Supplemental Table 2-6). Overall, 34 (23%) dust samples were positive for either Der f 1 or Der p 1 (Supplemental Table 2-7). Of the 28 homes with central air conditioning, 10 (36%) were positive for combined Der p 1 or Der f 1 in at least one location. Of the 20 homes with evaporative coolers, 11 (55%) were positive for combined Der p 1 or Der f 1 in at least one location. Only two homes were found with allergen concentrations (Der f 1 for both) that would be considered clinically significant for sensitization (i.e., > 2.0 µg/g dust). These samples were from a living room carpet (2.08 µg/g dust) and bedroom carpet (2.50 µg/g dust).

Although not statistically significant, the odds of Der f 1 concentrations above the detection limit were higher in homes with evaporative coolers compared to homes with central

air conditioning for all individual sample locations and all sample locations combined (Supplemental Table 2-5). Results were generally qualitatively similar for Der p 1 (Supplemental Table 2-6) and combined Der f 1 or Der p 1, but the association was statistically significant for combined Der f 1 or Der p 1 for all sample locations combined (OR = 2.29; 95% CI: 1.05, 4.98; P = 0.04) (Figure 1, Supplemental Table 2-7). Der f 1 concentration was significantly inversely associated with home size, but significantly positively associated with occupant density (Supplemental Table 2-8). Number of residents and occupant density were significantly positively associated with Der p 1 concentration (Supplemental Table 2-9) and number of residents, occupant density and bedroom carpet age were significantly positively associated with combined Der f 1 or Der p 1 concentration (Figure 1, Supplemental Table 2-10). Stepwise variable selection for Der f 1 selected home size. Adjusting the odds ratio for air conditioning type and Der f 1 for home size gave qualitatively similar results (OR = 2.06; 95% CI: 0.80, 5.32; P = 0.14). Stepwise variable selection for Der p 1 selected number of residents and bedroom mattress age. Adjusting the odds ratio for air conditioning type and Der p 1 for number of residents and bedroom mattress age gave qualitatively similar results (OR = 3.09; 95% CI: 0.85, 11.28; P = 0.09). Stepwise variable selection for combined Der f 1 or Der p 1 selected home size, number of residents and bedroom carpet age. Adjusting the odds ratio for air conditioning type and combined Der f 1 or Der p 1 for home size, number of residents and bedroom carpet age weakened the association (OR = 1.83; 95% CI: 0.70, 4.75; P = 0.22).

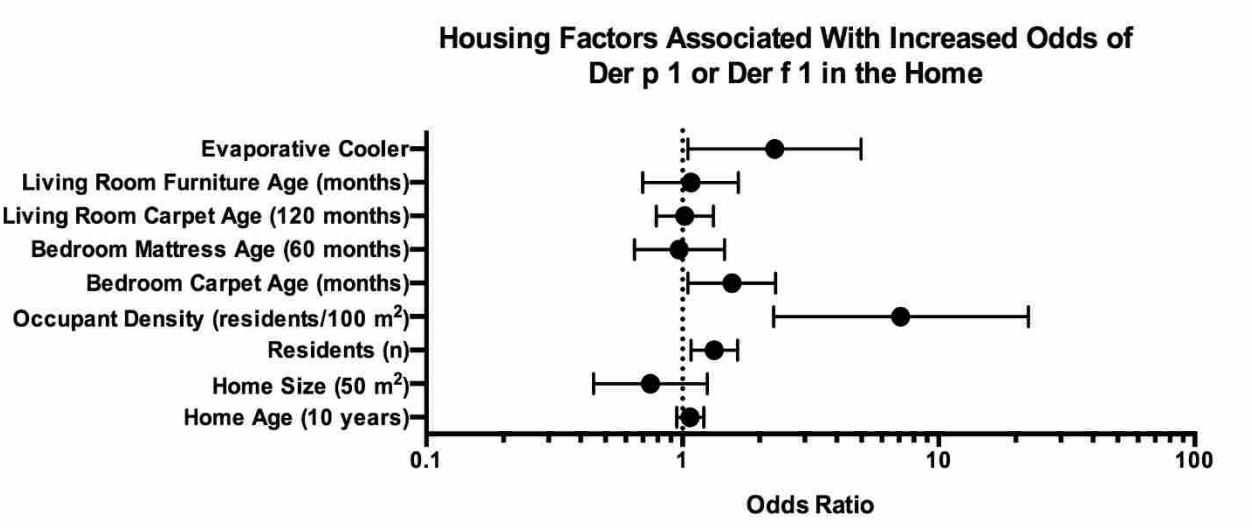

Figure 2-1. Housing factors associated with increased odds of Der p 1 or Der f 1 in the home. Odds ratio for evaporative cooler vs. central air conditioning or none was calculated using large sample/asymptotic approximate odds ratio (i.e. not exact). All other characteristics are exact odds ratios.

2.3.4 Endotoxin concentration (EU/mg) and surface load (EU/m^2)

Endotoxin concentrations were higher in homes with evaporative coolers compared to homes with central air conditioning for all individual sample locations and all sample locations combined, but these associations were statistically significant for only upholstered living room furniture samples (GM = 8.05 vs. 2.85 EU/mg, P = 0.0007) and all sample locations combined (GM = 3.60 vs. 1.29 EU/mg, P = 0.031) (Figure 2A, Supplemental Table 2-11). Results were qualitatively similar for endotoxin surface load (Figure 2B, Supplemental Table 2-12). Endotoxin concentration and surface load were significantly lower for bedroom mattress compared to bedroom carpet samples (Supplemental Tables 2-13-14). The multiple Tobit regression model for endotoxin concentration selected style of home and sample location. After adjusting for style of home and sample location, homes with evaporative coolers still had significantly higher endotoxin concentrations than homes with central air conditioning (P = 0.03). The multiple Tobit regression model for endotoxin surface load selected home age, home size, style of home and sample location. After adjusting for home age, home size, style of home

and sample location, air conditioning type was no longer significantly associated with endotoxin surface load (P = 0.09).

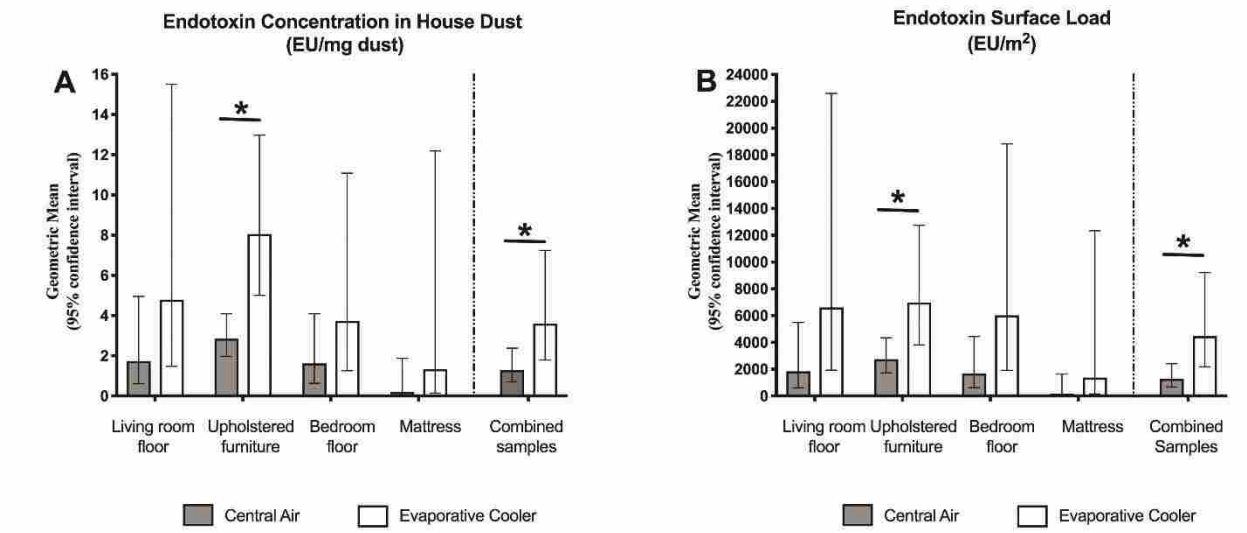

Figure 2-2. Endotoxin concentration and surface load in homes with central air conditioning or none and evaporative cooling. Mean endotoxin concentration (2-2A) and endotoxin surface load (2-2B) in homes with central air conditioning or none (n=28) and evaporative coolers (n=20) according to sampling location in the home (Living room floor, Upholstered furniture, Bedroom floor, Mattress). The mean of all of the samples (Combined samples) is also shown for endotoxin concentration and surface load. The histograms present geometric means and the error bars present 95% confidence intervals. * P<0.05

2.3.5 β-(1→3)-D-glucan concentration (µg/mg) and surface load (µg/m²)

β-(1→3)-D-glucan concentrations were significantly higher in homes with evaporative coolers compared to homes with central air conditioning for all individual sample locations and all sample locations combined (Figure 3A, Supplemental Table 2-15). Results were qualitatively similar for β-(1→3)-D-glucan surface load (Figure 3B, Supplemental Table 2-16). Home age, number of residents and bedroom carpet age were significantly associated with increased β-(1→3)-D-glucan concentration and surface load, whereas apartment versus two-story homes and living room furniture age were significantly associated with decreased β-(1→3)-D-glucan concentration and surface load (Supplemental Tables 2-17-18). Stepwise variable selection for β-(1→3)-D-glucan concentration selected number of residents, bedroom mattress age and living

room furniture age. After adjusting for number of residents, bedroom mattress age and living room furniture age, homes with evaporative coolers still had significantly higher β-(1→3)-D-glucan concentrations than homes with central air conditioning (P <0.0001). Stepwise variable selection for β-(1→3)-D-glucan surface load selected number of residents and living room furniture age. After adjusting for number of residents and living room furniture age, homes with evaporative coolers still had significantly higher β-(1→3)-D-glucan surface loads than homes with central air conditioning (P <0.0001).

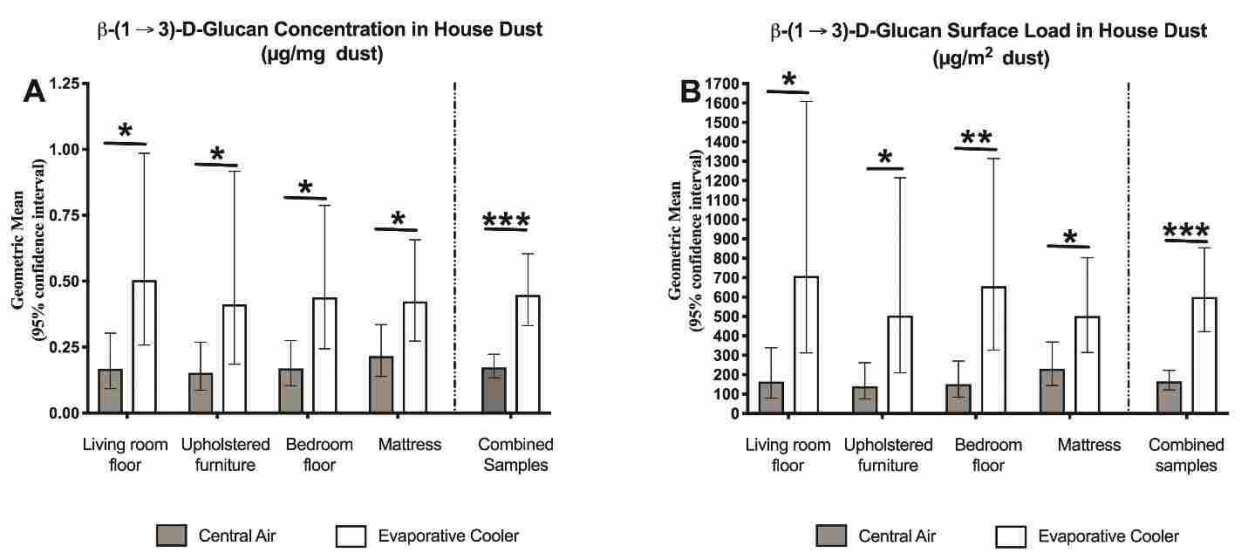

Figure 2-3. β-(1→3)-D-glucan concentration and surface load in homes with central air conditioning or none and evaporative cooling. Mean β-(1→3)-D-glucan concentration (2A) and β-(1→3)-D-glucan surface load (2B) in homes with central air conditioning or none (n=28) and evaporative coolers (n=20) according to sampling location in the home (Living room floor, Upholstered furniture, Bedroom floor, Mattress). The mean of all of the samples (Combined samples) is also shown for β-(1→3)-D-glucan concentration and surface load. The histograms present geometric means and the error bars present 95% confidence intervals. * P<0.05 **P<0.01 ***P<0.001

2.3.6 Spearman's rank correlation coefficients

Spearman's rank correlation coefficients generally indicated weak to moderate unadjusted associations between 72-hour mean RH and temperature, Der f 1, Der p 1, and combined Der f 1 or Der p 1 concentration, endotoxin concentration and surface load, and β-(1→3)-D-glucan concentration and surface load, although several of these associations were statistically significant (Supplemental Table 2-19).

2.4 Discussion

The prevalence of asthma and allergy sensitization in children has increased dramatically in recent decades, and the significant economic and quality of life impacts stemming from this has led to increased interest in understanding possible causes and prevention methods [110]. Human exposure to microbes and bioaerosols during different developmental windows and at different exposure levels can drive exacerbations, and paradoxically, can also play a protective role [111] in allergic diseases. Thus, improved understanding of environmental exposures, and the resultant effects, is paramount. Because children and adults spend on average between 60 – 80% of their time indoors at home, the home is a key microenvironment for exposure to bioaerosols. While previous studies have examined associations between house dust and indoor bioaerosols, only a few have examined the impact that residential cooling systems have on these levels. Our findings help clarify the role evaporative coolers play in indoor bioaerosol levels. In this study, we found significantly higher levels of combined Der p 1 or Der f 1, endotoxin, and β-(1→3)-D-glucan concentrations and surface loads in the dust of homes with evaporative cooling compared to those with central air conditioning. This study was performed during the summer months in low-income homes in a semi-arid climate (Utah County, Utah, USA) using four sample locations.

Prior studies from the Western U.S. and Australia report that children living in homes with evaporative coolers are more likely to be sensitized to HDM allergens [18], and homes with evaporative coolers are more likely to have clinically significant levels of Der p 1 and Der f 1 in settled dust [17,80]. The prevailing theory is that humidity from evaporative coolers provides enough moisture to indoor air to allow HDMs to survive in homes in dry climates. Findings from our study only partially support this theory. Both the prevalence and levels of Der p 1 and Der f 1 measured in our study homes are low compared to homes in more humid climates [112-114]. In fact,

only two samples in our study were above the threshold concentration (≥ 2.0 ug/g dust) for sensitization [115] A prior study of middle-income homes in Utah, and a study of mixed-income homes in rural western Montana and northern Idaho, both reported low levels of mite allergens in approximately 20 - 25% of homes, independent of air conditioning type [76,82] However, when homes are sampled according to socioeconomic status, as in this study, a different pattern emerges. For instance, a prior study of low-income homes with evaporative coolers in Utah [20] reported similarly low levels of mite allergens, but at a much higher prevalence (68%) than found in middle-income homes in the same community [76]. Our findings in this study were similar, with 55% of evaporative cooler homes testing positive for Der p 1 or Der f 1 in at least one location. Additionally, RH alone does not appear to explain this result.

An alternate theory supported by our data is that humidity from evaporative coolers works in tandem with occupant density and other housing factors to create an indoor environment that supports low levels of HDM populations in some dry climates, such as the Rocky Mountain States in the U.S. Our results show that evaporative coolers, increased number of residents, and older carpets were significantly associated with increased HDM allergen levels, but the characteristic with the strongest association was higher occupant density. Higher occupant densities in crowded, low-income housing may significantly increase indoor moisture through exhalation, cooking, bathing, and transfer of perspiration from bodies to upholstered furniture and mattresses. Higher occupant densities may lead to more people sitting and/or sleeping on mattresses and furniture, leading to more body moisture wicking into the fabric, and a higher concentration of shed skin cells, which provide a food source for HDMs. Older carpets may also serve as a reservoir for dead skin cells, which help support mite populations. The mean occupant density for homes in this study and the prior low-income study in Utah were 4.79 and

3.83 residents/100 m^2, respectively [20]. In contrast, occupant densities for middle-income homes in Utah were 1.72 residents/100 m^2 [76]. Thus, it appears that low RH in homes in dry climates, even when evaporative coolers are used, discourages dust mite growth unless there are compounding housing factors. Future studies on the relationship between evaporative coolers and mite allergens should consider housing factors and socioeconomic variables that may influence HDM growth and reproduction, particularly occupant density.

In this study, endotoxin concentrations were significantly higher in homes with evaporative coolers compared to homes with central air conditioning. Evaporative coolers had a 2.79-fold increase in endotoxin concentration levels (EU/mg), and a 3.49-fold increase in endotoxin surface load (EU/m^2) across the four sampling locations. Few studies have considered the influence that heating, ventilation, and air conditioning (HVAC) systems have on indoor endotoxin levels. Gereda et al. (2001), in a study of metropolitan homes in the Denver, CO area, found that central air conditioning was associated with lower endotoxin levels [99] Johnston et al. (2017), in a study of middle-income homes in Utah, reported endotoxin levels three to six times higher in homes with evaporative coolers compared to homes with central air conditioners [21]. Similarly, Lemons et al. (2017) found that endotoxin levels were approximately three times greater in homes with evaporative coolers compared to homes with central air conditioners in the Reno, NV area [71]. Although endotoxin levels were relatively low in our study, the observed proportional differences in endotoxin levels between the two types of air conditioning systems are consistent with Lemons et al. (2017) and Johnston et al. (2017), suggesting that evaporative coolers do tend to significantly increase residential endotoxin levels.

One plausible explanation for the higher levels of endotoxin found in homes with evaporative coolers is that Gram-negative bacteria grow in evaporative cooler sump water during

summer months, and the evaporative cooler distributes these bacteria, or their cell wall components, throughout the home. Macher & Girman (1990) found that Gram-negative bacteria grew to concentrations up to 1.4 x 10^5 cfu/ml over the course of the summer, and that these species were also recovered from indoor, but not outdoor, air [50]. However, this study was limited to a single home during the course of one summer. In contrast, Lemons et al. (2017) found no difference in airborne bacterial species between evaporative vs. central air conditioner homes [71]. Residential bioaerosols are often found on particles with aerodynamic diameters ranging from 0.1 – 10 μm, many of which are larger and settle out of the air quickly after resuspension [116,117]. Thus, many indoor bioaerosols may be underrepresented by stationary air samples. To better understand the influence evaporative coolers have on endotoxins and Gram-negative bacteria in the home, future research may consider comparing bacterial species from settled house dust in addition to air samples.

We used β-(1→3)-D-glucan as an omnibus measure of fungi in homes in this study and, to our knowledge, this is the first study to compare β-(1→3)-D-glucan levels among homes with evaporative and central air conditioning. β-(1→3)-D-glucan levels were significantly higher in evaporative cooling homes at all four sample locations. Evaporative coolers had a 2.64-fold increase in β-(1→3)-D-glucan concentration levels (μg/mg), and a 3.64-fold increase in β-(1→3)-D-glucan surface load (μg/m^2) across the four locations. Thus, of the three bioaerosols measured, β-(1→3)-D-glucan levels had the strongest and most consistent statistical increase in the evaporative cooling homes when compared to central air conditioning. In general, our findings are consistent with two prior studies that measured indoor fungi in homes with evaporative coolers. Sneller & Pinnas (1987) found significantly higher levels, and greater diversity, of fungal species in homes with evaporative coolers compared with central air

conditioners [101]. The most conclusive evidence to date is reported by Lemons et al. (2017), who found a higher prevalence of hydrophilic fungi in homes with evaporative coolers, including species in the order Pleosporales which are highly allergenic [71]. A likely explanation for our findings is that fungi grow in the sump water or cooling pads, and fungal spores are transported into the home during evaporative cooler operation. Lemons et al. found that species isolated from the sides of the water reservoir did not match with fungal species found in indoor air, and they suggest that the hydrophilic species found in indoor air originate from the cooling pads [71]. Future studies may clarify this issue by comparing fungal species found on evaporative cooler pads with those found in settled dust or air samples inside the home.

The role of residential exposure to mold in protecting or exacerbating asthma and allergy is not fully understood [118]. Certain types of fungi have been shown to be associated with protection from asthma and may also play a key role in influencing the composition of the microbiota and immune system [92,119]. Additional studies have identified no protective association between β-(1→3)-D-glucan exposure in household dust and health [120], while others have found it is associated with increased asthma prevalence in school aged children [121]. High levels of fungal exposure have been strongly implicated in the development and prevalence of asthma [122-124]. Whether or not fungal exposure is protective or harmful may depend upon the fungal species and the type and level of exposure. Fungal growth due to water damage in the home has been shown to be associated with increased levels of asthma [125] and low amounts of fungal diversity in house dust have been associated with asthma development [126]. Similar correlations between increases in asthma and a decrease in bacterial diversity have also been reported [127]. These findings are supportive of an extension of the hygiene hypothesis called the “biodiversity hypothesis” which takes into consideration the decrease in biodiversity of the microbes in our living environment as

risk factors for inflammatory diseases, in addition to the microbes in our food, water, and on domestic animals [27].

One critical issue that remains unresolved is how, when, and what type of microbial exposure is protective or harmful. Previous work has found protective effects of residential environmental exposure on the development of asthma and allergies. Children who have had high microbial exposures (increased numbers of siblings, grew up on a farm, frequent contact with farm animals) have been found to be at a reduced risk for asthma and allergies [43]. Exposure to coal or wood heating as children was also found to lower the risk for asthma, hay fever, and pollen sensitization [128]. In all of these cases, living in an environment as a child that is rich in microbial exposure, particularly lipopolysaccharide, was found to be protective [44,118]. Findings from this and other studies suggest that evaporative coolers play a significant role in changing the microbiological and bioaerosol profile of homes, although the immunological health benefits or risks associated with these changes are not fully understood.

It is possible that early-life exposure to microbes and bioaerosols from evaporative coolers could confer protection against allergies and asthma, consistent with the hygiene hypothesis [29,84]. Recent findings from a large pediatric cohort study in Colorado found no association between evaporative cooler use and dust mite or mold sensitization when comparing children living in homes with evaporative coolers vs. central air conditioners. Interestingly, they did report significantly lower prevalence of allergic rhinitis among children living in homes with evaporative coolers [19]. Conversely, other studies show that children living in homes with evaporative coolers are more likely to test positive for sensitization to mold and HDM allergens [18], and are more likely to experience lower respiratory tract illness in infancy [129]. While our study does not directly address health outcomes, it is a relevant issue that needs to be addressed when

considering whether or not to develop or keep policies that provide financial incentives to get or replace evaporative coolers.

Although selecting our study homes based on household income allowed us to control for socioeconomic factors, this study was limited by a relatively small sample size drawn from one community. Thus, our results may not be generalizable to homes in other locations. Due to logistical challenges and the fact that evaporative coolers are only used for a few months out of the year, we did not complete our sample collection until October, 2017. Homes in the final weeks of sample collection, which included several evaporative cooler homes, had likely stopped using their air conditioners for the year. This likely explained why we did not see a difference in RH levels between evaporative cooler and air conditioner homes.

In conclusion, we found significantly higher levels of combined Der p 1 or Der f 1, endotoxin, and β-(1→3)-D-glucans in low-income homes with evaporative coolers when compared to homes with central air conditioning. Based on this and other studies, it appears that evaporative coolers have a significant and potentially important impact on the microbiological and bioaerosol profile of homes in dry climates. There are several questions that remain unanswered regarding the health benefits or risks associated with evaporative coolers, which should be addressed by future studies.

2.5 Acknowledgements

We would like to thank the Woman, Infants, and Children (WIC) office at the Utah County Health Department (UCHD) for their support in recruiting participants for this study. We are also indebted to the gracious individuals who participated in this study for allowing us into their homes to collect our samples. This research was funded by the Ira and Mary Lou Fulton Gift Fund at Brigham Young University. The authors have no conflicts of interest to declare.

Supplemental tables

Supplemental Table 2-1. House relative humidity in low-income homes in Utah County, Utah, Summer 2017.

House 72-hour Mean Relative Humidity	n (%)	AM†	95% CI†	Min	Max	p-value†
72-hour mean relative humidity, %	48 (100)	44.18	41.49, 46.86	25.39	71.58	
Central air conditioning‡	28 (58)	43.91	40.36, 47.47	27.11	71.58	
Evaporative cooler	20 (42)	44.54	40.34, 48.74	25.39	65.35	0.82

Abbreviations: AM, arithmetic mean; CI, confidence interval; Min, minimum; Max, maximum.
† Estimated via linear regression models of the original values.
‡ Includes 24 homes that had central air conditioning and four homes that had no air conditioning.

Supplemental Table 2-2. Characteristics and house relative humidity of low-income homes in Utah County, Utah, Summer 2017.

	House 72-hour Mean Relative Humidity		
Characteristic	n (%)	Missing, n	Unadjusted β† (95% CI†)
Home age, 10 years		1	0.11 (-0.83, 1.04)
Home size, 50 m^2		1	-1.94 (-5.20, 1.31)
Style of home, n (%)			
Apartment	12 (25)		2.11 (-5.43, 9.65)
Single story	8 (17)		-0.39 (-8.85, 8.07)
Two story	13 (27)		Reference
Other‡	15 (31)		4.29 (-2.85, 11.43)
Residents, n			0.04 (-1.46, 1.54)
Occupant density, residents/100 m^2§		1	2.94 (-3.22, 9.09)
Bedroom carpet age, months§		5	-1.60 (-3.60, 0.41)
Bedroom mattress age, 60 months			-0.87 (-3.88, 2.14)
Living room carpet age, 120 months		6	-1.07 (-2.78, 0.64)
Living room furniture age, months§			1.05 (-1.91, 4.01)

Abbreviations: CI, confidence interval.
† Estimated via linear regression models of the original values.
‡ Includes basement apartment (seven dust samples), condo (three dust samples), duplex (nine dust samples), four plex (three dust samples), four plex split level (two dust samples), split entry (five dust samples), trailer (two samples), tri-level split (four samples), and not applicable (six dust samples).
§ Transformed by taking the natural logarithm of the original values

Supplemental Table 2-3. House temperature in low-income homes in Utah County, Utah, Summer 2017.

House 72-hour Mean Temperature	n (%)	AM[†]	95% CI[†]	Min	Max	p-value[†]
72-hour mean temperature, °C	48 (100)	24.04	23.56, 24.52	21.12	28.00	
Central air conditioning[‡]	28 (58)	24.56	23.97, 25.14	21.84	28.00	
Evaporative cooler	20 (42)	23.31	22.62, 24.00	21.12	26.37	0.008

Abbreviations: AM, arithmetic mean; CI, confidence interval; Min, minimum; Max, maximum.
[†] Estimated via linear regression models of the original values.
[‡] Includes 24 homes that had central air conditioning and four homes that had no air conditioning

Supplemental Table 2-4. Characteristics and house temperature of low-income homes in Utah County, Utah, Summer 2017.

	House 72-hour Mean Temperature		
Characteristic	n (%)	Missing, n	Unadjusted β[†] (95% CI[†])
Home age, 10 years		1	-0.01 (-0.18, 0.16)
Home size, 50 m^2		1	0.06 (-0.54, 0.67)
Style of home, n (%)			
Apartment	12 (25)		0.20 (-1.16, 1.55)
Single story	8 (17)		-0.50 (-2.02, 1.02)
Two story	13 (27)		Reference
Other[‡]	15 (31)		0.18 (-1.11, 1.46)
Residents, n			<0.01 (-0.26, 0.27)
Occupant density, residents/100 $m^{2§}$		1	0.23 (-0.91, 1.37)
Bedroom carpet age, months[§]		5	0.13 (-0.25, 0.51)
Bedroom mattress age, 60 months			-0.23 (-0.76, 0.31)
Living room carpet age, 120 months		6	0.16 (-0.15, 0.47)
Living room furniture age, months[§]			-0.16 (-0.69, 0.37)

Abbreviations: CI, confidence interval.
[†] Estimated via linear regression models of the original values.
[‡] Includes basement apartment (seven dust samples), condo (three dust samples), duplex (nine dust samples), four plex (three dust samples), four plex split level (two dust samples), split entry (five dust samples), trailer (two samples), tri-level split (four samples), and not applicable (six dust samples).
[§] Transformed by taking the natural logarithm of the original value

Supplemental Table 2-5. House dust mite allergen (Der f 1) concentration in low-income homes in Utah County, Utah, Summer 2017.

House Dust Mite Allergen Concentration	Negative[†,‡], n (%)	Positive[†,‡]					Unadjusted Exact OR (Exact 95% CI)
		n (%)	GM	GSD	Min	Max	
Der f 1, µg/g of dust	129 (85)	22 (15)	0.16	3.61	0.04	2.50	
Central air conditioning[§]	77 (90)	9 (10)	0.11	2.09	0.04	0.43	Reference
Evaporative cooler	52 (80)	13 (20)	0.20	4.63	0.04	2.50	2.14 (0.85, 5.37)[¶]
Bedroom carpet							
Central air conditioning[§]	22 (85)	4 (15)	0.11	2.64	0.04	0.43	Reference
Evaporative cooler	15 (75)	5 (25)	0.43	4.29	0.06	2.50	1.81 (0.33, 10.74)
Bedroom mattress							
Central air conditioning[§]	15 (94)	1 (6)	0.12	N/A	0.12	0.12	Reference
Evaporative cooler	13 (81)	3 (19)	0.09	2.90	0.04	0.30	3.34 (0.23, 193.60)
Living room carpet							
Central air conditioning[§]	21 (88)	3 (13)	0.08	1.56	0.05	0.12	Reference
Evaporative cooler	15 (79)	4 (21)	0.22	6.15	0.05	2.08	1.84 (0.27, 14.46)
Living room furniture							
Central air conditioning[§]	19 (95)	1 (5)	0.26	N/A	0.26	0.26	Reference
Evaporative cooler	9 (90)	1 (10)	0.04	N/A	0.04	0.04	2.06 (0.02, 174.28)

Abbreviations: CI, confidence interval; Der f 1, *Dermatophagoides farinae*; GM, geometric mean; GSD, geometric standard deviation; Min, minimum; Max, maximum; N/A, not applicable; OR, odds ratio.

[†] 41 dust samples did not have enough dust in which to measure dust mite allergen concentration.

[‡] The detection limit was 0.04 µg/g of dust.

[§] Includes 24 homes (76 dust samples) that had central air conditioning and four homes (10 dust samples) that had no air conditioning.

[¶] Large sample/asymptotic approximate OR (large sample/asymptotic approximate 95% CI) (i.e., not exact).

Supplemental Table 2-6. House dust mite allergen (Der p 1) concentration in low-income homes in Utah County, Utah, Summer 2017.

House Dust Mite Allergen Concentration	Negative[†,‡], n (%)	Positive[†,‡]					Unadjusted Exact OR (Exact 95% CI)
		n (%)	GM	GSD	Min	Max	
Der p 1, µg/g of dust	135 (89)	16 (11)	0.13	2.60	0.04	0.65	
Central air conditioning[§]	80 (93)	6 (7)	0.08	2.03	0.04	0.27	Reference
Evaporative cooler	55 (85)	10 (15)	0.17	2.75	0.04	0.65	2.42 (0.83, 7.06)[¶]
Bedroom carpet							
Central air conditioning[§]	25 (96)	1 (4)	0.04	N/A	0.04	0.04	Reference
Evaporative cooler	18 (90)	2 (10)	0.15	1.67	0.11	0.22	2.72 (0.13, 170.31)
Bedroom mattress							
Central air conditioning[§]	14 (88)	2 (13)	0.12	3.24	0.05	0.27	Reference
Evaporative cooler	13 (81)	3 (19)	0.16	2.32	0.06	0.28	1.59 (0.16, 21.95)
Living room carpet							
Central air conditioning[§]	24 (100)	0 (0)	N/A	N/A	N/A	N/A	Reference
Evaporative cooler	15 (79)	4 (21)	0.13	3.82	0.04	0.61	7.74 (1.24, ∞)[††]
Living room furniture							
Central air conditioning[§]	17 (85)	3 (15)	0.08	1.62	0.05	0.13	Reference
Evaporative cooler	9 (90)	1 (10)	0.65	N/A	0.65	0.65	0.64 (0.01, 9.38)

Abbreviations: CI, confidence interval; Der p 1, *Dermatophagoides pteronyssinus*; GM, geometric mean; GSD, geometric standard deviation; Min, minimum; Max, maximum; N/A, not applicable; OR, odds ratio.

[†] 41 dust samples did not have enough dust in which to measure dust mite allergen concentration.

[‡] The detection limit was 0.04 µg/g of dust.

[§] Includes 24 homes (76 dust samples) that had central air conditioning and four homes (10 dust samples) that had no air conditioning.

[¶] Large sample/asymptotic approximate OR (large sample/asymptotic approximate 95% CI) (i.e., not exact).

[††] Median unbiased estimate.

Supplemental Table 2-7. House dust mite allergen (combined Der f 1 or Der p 1) concentration in low-income homes in Utah County, Utah, Summer 2017.

House Dust Mite Allergen Concentration	Negative[†,‡], n (%)	Positive[†,‡]					Unadjusted Exact OR (Exact 95% CI)
		n (%)	GM	GSD	Min	Max	
Combined Der f 1 or Der p 1, µg/g of dust	117 (77)	34 (23)	0.15	3.15	0.04	2.50	
Central air conditioning[§]	72 (84)	14 (16)	0.09	2.03	0.04	0.43	Reference
Evaporative cooler	45 (69)	20 (31)	0.21	3.64	0.04	2.50	2.29 (1.05, 4.98)[¶]
Bedroom carpet							
Central air conditioning[§]	21 (81)	5 (19)	0.09	2.56	0.04	0.43	Reference
Evaporative cooler	14 (70)	6 (30)	0.35	3.78	0.06	2.50	1.80 (0.46, 7.06)[¶]
Bedroom mattress							
Central air conditioning[§]	14 (88)	2 (13)	0.10	2.58	0.05	0.19	Reference
Evaporative cooler	11 (69)	5 (31)	0.14	2.45	0.05	0.30	3.07 (0.40, 38.12)
Living room carpet							
Central air conditioning[§]	21 (88)	3 (13)	0.08	1.56	0.05	0.12	Reference
Evaporative cooler	12 (63)	7 (37)	0.19	4.40	0.04	2.08	3.95 (0.73, 28.14)
Living room furniture							
Central air conditioning[§]	16 (80)	4 (20)	0.11	2.04	0.05	0.26	Reference
Evaporative cooler	8 (80)	2 (20)	0.16	7.21	0.04	0.65	1.00 (0.08, 8.88)

Abbreviations: CI, confidence interval; Der f 1, *Dermatophagoides farinae*; Der p 1, *Dermatophagoides pteronyssinus*; GM, geometric mean; GSD, geometric standard deviation; Min, minimum; Max, maximum; N/A, not applicable; OR, odds ratio.

[†] 41 dust samples did not have enough dust in which to measure dust mite allergen concentration.

[‡] The detection limit was 0.04 µg/g of dust.

[§] Includes 24 homes (76 dust samples) that had central air conditioning and four homes (10 dust samples) that had no air conditioning.

[¶] Large sample/asymptotic approximate OR (large sample/asymptotic approximate 95% CI) (i.e., not exact).

Supplemental Table 2-8. Characteristics and house dust mite allergen (Der f 1) concentration of low-income homes in Utah County, Utah, Summer 2017.

	House Dust Mite Allergen (Der f 1) Concentration[†]			
Characteristic	Negative[‡], n (%)	Positive[‡], n (%)	Missing, n	Unadjusted OR (95% CI)
Home age, 10 years			4	0.98 (0.84, 1.15)
Home size, 50 m^2			4	0.30 (0.12, 0.74)
Style of home, n (%)				
Apartment	31 (24)	9 (41)		3.81 (0.86, 23.71)[§]
Single story	22 (17)	4 (18)		2.39 (0.37, 17.83)[§]
Two story	40 (31)	3 (14)		Reference
Other[¶]	36 (28)	6 (27)		2.20 (0.43, 14.60)[§]
Residents, n				0.90 (0.69, 1.17)
Occupant density, residents/100 $m^{2††}$			4	4.47 (1.28, 15.59)
Bedroom carpet age, months[††]			16	1.36 (0.86, 2.16)
Bedroom mattress age, 60 months				1.17 (0.75, 1.82)
Living room carpet age, 120 months			18	1.11 (0.84, 1.47)
Living room furniture age, months[††]				0.79 (0.49, 1.28)
Sample location, n (%)				
Bedroom carpet	37 (29)	9 (41)		Reference
Bedroom mattress	28 (22)	4 (18)		0.59 (0.12, 2.39)[§]
Living room carpet	36 (28)	7 (32)		0.80 (0.23, 2.72)[§]
Living room furniture	28 (22)	2 (9)		0.30 (0.03, 1.60)[§]

Abbreviations: CI, confidence interval; Der f 1, *Dermatophagoides farinae*; OR, odds ratio.

[†] 41 dust samples did not have enough dust in which to measure dust mite allergen concentration.

[‡] The detection limit was 0.04 μg/g of dust.

[§] Exact OR (exact 95% CI) (i.e., not large sample/asymptotic approximate).

[¶] Includes basement apartment (seven dust samples), condo (three dust samples), duplex (nine dust samples), four plex (three dust samples), four plex split level (two dust samples), split entry (five dust samples), trailer (three samples), tri-level split (four samples), and not applicable (six dust samples).

[††] Transformed by taking the natural logarithm of the original values.

Supplemental Table 2-9. Characteristics and house dust mite allergen (Der p 1) concentration of low-income homes in Utah County, Utah, Summer 2017.

	House Dust Mite Allergen (Der p 1) Concentration[†]			
Characteristic	Negative[‡], n (%)	Positive[‡], n (%)	Missing, n	Unadjusted OR (95% CI)
Home age, 10 years			4	1.14 (0.97, 1.33)
Home size, 50 m^2			4	1.49 (0.88, 2.52)
Style of home, n (%)				
Apartment	38 (28)	2 (13)		0.33 (0.03, 1.99)[§]
Single story	19 (14)	7 (44)		2.24 (0.56, 9.36)[§]
Two story	37 (27)	6 (38)		Reference
Other[¶]	41 (30)	1 (6)		0.15 (<0.01, 1.35)[§]
Residents, n				2.03 (1.45, 2.86)
Occupant density, residents/100 m^2[††]			4	6.45 (1.50, 27.79)
Bedroom carpet age, months[††]			16	1.39 (0.88, 2.19)
Bedroom mattress age, 60 months				0.49 (0.21. 1.14)
Living room carpet age, 120 months			18	0.80 (0.49, 1.31)
Living room furniture age, months[††]				1.21 (0.67, 2.20)
Sample location, n (%)				
Bedroom carpet	43 (32)	3 (19)		Reference
Bedroom mattress	27 (20)	5 (31)		2.62 (0.47, 18.25)[§]
Living room carpet	39 (29)	4 (25)		1.46 (0.23, 10.63)[§]
Living room furniture	26 (19)	4 (25)		2.18 (0.34, 16.09)[§]

Abbreviations: CI, confidence interval; Der p 1, *Dermatophagoides pteronyssinus*; OR, odds ratio.

[†] 41 dust samples did not have enough dust in which to measure dust mite allergen concentration.

[‡] The detection limit was 0.04 µg/g of dust.

[§] Exact OR (exact 95% CI) (i.e., not large sample/asymptotic approximate).

[¶] Includes basement apartment (seven dust samples), condo (three dust samples), duplex (nine dust samples), four plex (three dust samples), four plex split level (two dust samples), split entry (five dust samples), trailer (three samples), tri-level split (four samples), and not applicable (six dust samples).

[††] Transformed by taking the natural logarithm of the original values.

Supplemental Table 2-10. Characteristics and house dust mite allergen (combined Der f 1 or Der p 1) concentration of low-income homes in Utah County, Utah, Summer 2017.

	House Dust Mite Allergen (Combined Der f 1 or Der p 1) Concentration[†]			
Characteristic	Negative[‡], n (%)	Positive[‡], n (%)	Missing, n	Unadjusted OR (95% CI)
Home age, 10 years			4	1.07 (0.95, 1.21)
Home size, 50 m^2			4	0.75 (0.45, 1.25)
Style of home, n (%)				
Apartment	30 (26)	10 (29)		1.46 (0.51, 4.17)
Single story	17 (15)	9 (26)		2.29 (0.65, 8.20)
Two story	35 (30)	8 (24)		Reference
Other[§]	35 (30)	7 (21)		0.88 (0.24, 3.11)
Residents, n				1.33 (1.08, 1.64)
Occupant density, residents/100 m^2[¶]			4	7.10 (2.27, 22.42)
Bedroom carpet age, months[¶]			16	1.56 (1.05, 2.31)
Bedroom mattress age, 60 months				0.97 (0.65, 1.46)
Living room carpet age, 120 months			18	1.02 (0.79, 1.32)
Living room furniture age, months[¶]				1.08 (0.70, 1.65)
Sample location, n (%)				
Bedroom carpet	35 (30)	11 (32)		Reference
Bedroom mattress	25 (21)	7 (21)		0.89 (0.30, 2.62)
Living room carpet	33 (28)	10 (29)		0.96 (0.36, 2.57)
Living room furniture	24 (21)	6 (18)		0.80 (0.26, 2.44)

Abbreviations: CI, confidence interval; Der f 1, *Dermatophagoides farinae*; Der p 1, *Dermatophagoides pteronyssinus*; OR, odds ratio.

[†] 41 dust samples did not have enough dust in which to measure dust mite allergen concentration.

[‡] The detection limit was 0.04 μg/g of dust.

[§] Includes basement apartment (seven dust samples), condo (three dust samples), duplex (nine dust samples), four plex (three dust samples), four plex split level (two dust samples), split entry (five dust samples), trailer (three samples), tri-level split (four samples), and not applicable (six dust samples).

[¶] Transformed by taking the natural logarithm of the original values.

Supplemental Table 2-11. House dust endotoxin concentration in low-income homes in Utah County, Utah, Summer 2017.

House Dust Endotoxin Concentration	Negative[†,‡], n (%)	Positive[†,‡]					p-value[§]
		n (%)	GM[§]	95% CI[§]	Min[¶]	Max[¶]	
Endotoxin, units/mg of dust	9 (6)	138 (94)	2.01	1.26, 3.22	0.71	53.25	
Central air conditioning[††]	5 (6)	78 (94)	1.29	0.70, 2.38	0.71	37.56	
Evaporative cooler	4 (6)	60 (94)	3.60	1.79, 7.24	2.07	53.25	0.03
Bedroom carpet							
Central air conditioning[††]	1 (4)	25 (96)	1.62	0.64, 4.10	0.80	37.56	
Evaporative cooler	1 (5)	18 (95)	3.74	1.26, 11.09	3.45	50.01	0.25
Bedroom mattress							
Central air conditioning[††]	3 (19)	13 (81)	0.20	0.02, 1.88	0.71	14.08	
Evaporative cooler	2 (13)	14 (88)	1.34	0.15, 12.19	2.21	17.78	0.24
Living room carpet							
Central air conditioning[††]	1 (4)	23 (96)	1.74	0.61, 4.95	0.77	36.43	
Evaporative cooler	1 (5)	18 (95)	4.79	1.48, 15.51	2.07	53.25	0.21
Living room furniture							
Central air conditioning[††]	0 (0)	17 (100)	2.85	1.97, 4.10	1.01	11.18	
Evaporative cooler	0 (0)	10 (100)	8.05	5.00, 12.98	2.42	15.60	0.0007

Abbreviations: CI, confidence interval; GM, geometric mean; Min, minimum; Max, maximum.

[†] 45 dust samples did not have enough dust in which to measure endotoxin concentration.

[‡] The detection limit was 0.0001 endotoxin units/mg of dust.

[§] Estimated via Tobit regression models of the natural logarithm transformed values.

[¶] Calculated from positive samples only.

[††] Includes 24 homes (73 dust samples) that had central air conditioning and four homes (10 dust samples) that had no air conditioning.

Supplemental Table 2-12. House dust endotoxin surface load in low-income homes in Utah County, Utah, Summer 2017.

		Positive[†,‡]					
House Dust Endotoxin Surface Load	Negative[†,‡], n (%)	n (%)	GM[§]	95% CI[§]	Min[¶]	Max[¶]	p-value[§]
Endotoxin, units/m^2	9 (6)	138 (94)	2,205.22	1,354.23, 3,590.99	387.60	117,468.79	
Central air conditioning[††]	5 (6)	78 (94)	1,281.99	680.53, 2,415.03	387.60	56,524.41	
Evaporative cooler	4 (6)	60 (94)	4,476.54	2,176.81, 9,205.87	1,064.54	117,468.79	0.01
Bedroom carpet							
Central air conditioning[††]	1 (4)	25 (96)	1,678.76	633.79, 4,446.59	820.55	56,367.92	
Evaporative cooler	1 (5)	18 (95)	6,018.47	1,925.54, 18,811.37	2,770.03	117,468.79	0.10
Bedroom mattress							
Central air conditioning[††]	3 (19)	13 (81)	179.40	19.60, 1,642.47	409.12	7,317.81	
Evaporative cooler	2 (13)	14 (88)	1,371.25	152.34, 12,342.62	1,865.75	31,293.03	0.20
Living room carpet							
Central air conditioning[††]	1 (4)	23 (96)	1,843.02	617.40, 5,501.65	387.60	56,524.41	
Evaporative cooler	1 (5)	18 (95)	6,610.06	1,933.57, 22,597.06	1,671.51	98,739.80	0.13
Living room furniture							
Central air conditioning[††]	0 (0)	17 (100)	2,740.14	1,726.48, 4,348.92	758.73	12,103.91	
Evaporative cooler	0 (0)	10 (100)	6,975.90	3,819.77, 12,739.82	1,064.54	43,221.02	0.02

Abbreviations: CI, confidence interval; GM, geometric mean; Min, minimum; Max, maximum.

[†] 45 dust samples did not have enough dust in which to measure endotoxin surface load.

[‡] The detection limit was 0.0001 endotoxin units/mg of dust.

[§] Estimated via Tobit regression models of the natural logarithm transformed values.

[¶] Calculated from positive samples only.

[††] Includes 24 homes (73 dust samples) that had central air conditioning and four homes (10 dust samples) that had no air conditioning.

Supplemental Table 2-13. Characteristics and house dust endotoxin concentration of low-income homes in Utah County, Utah, Summer 2017.

	House Dust Endotoxin Concentration[†]			
Characteristic	Negative[‡], n (%)	Positive[‡], n (%)	Missing, n	Unadjusted exp(β)[§] (95% CI[§])
Home age, 10 years			4	1.05 (0.89, 1.23)
Home size, 50 m^2			4	0.65 (0.37, 1.15)
Style of home, n (%)				
Apartment	0 (0)	38 (28)		2.86 (0.81, 10.03)
Single story	1 (11)	25 (18)		2.85 (0.70, 11.56)
Two story	4 (44)	38 (28)		Reference
Other[¶]	4 (44)	37 (27)		1.13 (0.33, 3.88)
Residents, n				1.07 (0.83, 1.38)
Occupant density, residents/100 m^2[††]			4	2.46 (0.81, 7.51)
Bedroom carpet age, months[††]			14	1.21 (0.86, 1.69)
Bedroom mattress age, 60 months				1.08 (0.66, 1.78)
Living room carpet age, 120 months			16	1.09 (0.80, 1.48)
Living room furniture age, months[††]				0.88 (0.53, 1.46)
Sample location, n (%)				
Bedroom carpet	2 (22)	43 (31)		Reference
Bedroom mattress	5 (56)	27 (20)		0.29 (0.07, 0.96)
Living room carpet	2 (22)	41 (30)		1.18 (0.36, 3.86)
Living room furniture	0 (0)	27 (20)		1.83 (0.47, 7.03)

Abbreviations: CI, confidence interval.

[†] 45 dust samples did not have enough dust in which to measure endotoxin concentration.

[‡] The detection limit was 0.0001 endotoxin units/mg of dust.

[§] Estimated via Tobit regression models of the natural logarithm transformed values. Exponentiated regression coefficient and 95% CI is the geometric mean house dust endotoxin concentration ratio for a specified change in the independent variable or exp(β) – 1 = percent change in geometric mean house dust endotoxin concentration for a specified change in the independent variable.

[¶] Includes basement apartment (seven dust samples), condo (two dust samples), duplex (nine dust samples), four plex (three dust samples), four plex split level (two dust samples), split entry (five dust samples), trailer (three samples), tri-level split (four samples), and not applicable (six dust samples).

[††] Transformed by taking the natural logarithm of the original values.

Supplemental Table 2-14. Characteristics and house dust endotoxin surface load of low-income homes in Utah County, Utah, Summer 2017.

	House Dust Endotoxin Surface Load[†]			
Characteristic	Negative[‡], n (%)	Positive[‡], n (%)	Missing, n	Unadjusted exp(β)[§] (95% CI[§])
Home age, 10 years			4	1.10 (0.93, 1.30)
Home size, 50 m^2			4	0.67 (0.37, 1.21)
Style of home, n (%)				
Apartment	0 (0)	38 (28)		2.32 (0.63, 8.55)
Single story	1 (11)	25 (18)		3.18 (0.74, 13.67)
Two story	4 (44)	38 (28)		Reference
Other[¶]	4 (44)	37 (27)		1.10 (0.31, 3.97)
Residents, n				1.13 (0.87, 1.46)
Occupant density, residents/100 m^2[††]			4	2.83 (0.89, 8.99)
Bedroom carpet age, months[††]			14	1.24 (0.88, 1.76)
Bedroom mattress age, 60 months				1.05 (0.63, 1.76)
Living room carpet age, 120 months			16	1.07 (0.78, 1.48)
Living room furniture age, months[††]				0.81 (0.48, 1.38)
Sample location, n (%)				
Bedroom carpet	2 (22)	43 (31)		Reference
Bedroom mattress	5 (56)	27 (20)		0.20 (0.05, 0.76)
Living room carpet	2 (22)	41 (30)		1.13 (0.33, 3.84)
Living room furniture	0 (0)	27 (20)		1.36 (0.34, 5.47)

Abbreviations: CI, confidence interval.

[†] 45 dust samples did not have enough dust in which to measure endotoxin surface load.

[‡] The detection limit was 0.0001 endotoxin units/mg of dust.

[§] Estimated via Tobit regression models of the natural logarithm transformed values. Exponentiated regression coefficient and 95% CI is the geometric mean house dust endotoxin surface load ratio for a specified change in the independent variable or exp(β) – 1 = percent change in geometric mean house dust endotoxin surface load for a specified change in the independent variable.

[¶] Includes basement apartment (seven dust samples), condo (two dust samples), duplex (nine dust samples), four plex (three dust samples), four plex split level (two dust samples), split entry (five dust samples), trailer (three samples), tri-level split (four samples), and not applicable (six dust samples).

[††] Transformed by taking the natural logarithm of the original values.

Supplemental Table 2-15. House dust β-(1→3)-D-glucan concentration in low-income homes in Utah County, Utah, Summer 2017.

House Dust β-(1→3)-D-glucan Concentration	n (%)[†, ‡]	GM[§]	95% CI[§]	Min	Max	p-value[§]
β-(1→3)-D-glucan, μg/mg of dust	147 (100)	0.26	0.21, 0.32	0.0031	2.20	
Central air conditioning[¶]	85 (58)	0.17	0.13, 0.22	0.0031	2.01	
Evaporative cooler	62 (42)	0.45	0.33, 0.60	0.09	2.20	<0.0001
Bedroom carpet						
Central air conditioning[¶]	26 (18)	0.17	0.10, 0.28	0.0031	1.57	
Evaporative cooler	18 (12)	0.44	0.24, 0.79	0.09	1.21	0.02
Bedroom mattress						
Central air conditioning[¶]	16 (11)	0.22	0.14, 0.34	0.071	1.03	
Evaporative cooler	16 (11)	0.42	0.27, 0.66	0.15	2.20	0.04
Living room carpet						
Central air conditioning[¶]	23 (16)	0.17	0.093, 0.30	0.0066	1.76	
Evaporative cooler	18 (12)	0.50	0.26, 0.99	0.11	1.60	0.02
Living room furniture						
Central air conditioning[¶]	20 (14)	0.15	0.087, 0.27	0.0034	2.01	
Evaporative cooler	10 (7)	0.41	0.18, 0.92	0.099	0.85	0.05

Abbreviations: CI, confidence interval; GM, geometric mean; Min, minimum; Max, maximum.

[†] 45 dust samples did not have enough dust in which to measure β-(1→3)-D-glucan concentration.

[‡] The detection limit was 0.0000625 μg/mg of dust.

[§] Estimated via linear regression models of the natural logarithm transformed values.

[¶] Includes 24 homes (75 dust samples) that had central air conditioning and four homes (10 dust samples) that had no air conditioning.

Supplemental Table 2-16. House dust β-(1→3)-D-glucan surface load in low-income homes in Utah County, Utah, Summer 2017.

House Dust β-(1→3)-D-glucan Surface Load	n (%)[†, ‡]	GM[§]	95% CI[§]	Min	Max	p-value[§]
β-(1→3)-D-glucan, μg/m^2	147 (100)	284.13	221.09, 365.16	2.10	3531.92	
Central air conditioning[¶]	85 (58)	164.69	121.88, 222.53	2.10	3531.92	
Evaporative cooler	62 (42)	600.14	421.87, 853.74	63.93	2844.91	<0.0001
Bedroom carpet						
Central air conditioning[¶]	26 (18)	151.40	84.82, 270.24	2.10	2357.25	
Evaporative cooler	18 (12)	655.29	326.60, 1314.77	73.16	2844.91	0.002
Bedroom mattress						
Central air conditioning[¶]	16 (11)	230.99	144.60, 369.01	51.77	1319.85	
Evaporative cooler	16 (11)	502.16	314.34, 802.20	115.36	2242.55	0.02
Living room carpet						
Central air conditioning[¶]	23 (16)	164.26	79.58, 339.03	2.51	3531.92	
Evaporative cooler	18 (12)	708.98	312.52, 1608.40	63.93	2527.37	0.01
Living room furniture						
Central air conditioning[¶]	20 (14)	140.59	75.52, 261.70	3.60	2022.89	
Evaporative cooler	10 (7)	504.78	209.63, 1215.50	79.39	2111.29	0.02

Abbreviations: CI, confidence interval; GM, geometric mean; Min, minimum; Max, maximum.

[†] 45 dust samples did not have enough dust in which to measure β-(1→3)-D-glucan surface load.

[‡] The detection limit was 0.0000625 μg/mg of dust.

[§] Estimated via linear regression models of the natural logarithm transformed values.

[¶] Includes 24 homes (75 dust samples) that had central air conditioning and four homes (10 dust samples) that had no air conditioning.

Supplemental Table 2-17. Characteristics and house dust β-(1→3)-D-glucan concentration of low-income homes in Utah County, Utah, Summer 2017.

	House Dust β-(1→3)-D-glucan Concentration[†]		
Characteristic	n (%)[‡]	Missing, n	Unadjusted exp(β)[§] (95% CI[§])
Home age, 10 years		4	1.08 (1.01, 1.16)
Home size, 50 m^2		4	1.27 (0.99, 1.63)
Style of home, n (%)			
Apartment	39 (27)		0.48 (0.28, 0.83)
Single story	24 (16)		1.78 (0.96, 3.30)
Two story	43 (29)		Reference
Other[¶]	41 (28)		0.91 (0.54, 1.54)
Residents, n			1.24 (1.12, 1.38)
Occupant density, residents/100 $m^{2††}$		4	1.41 (0.86, 2.32)
Bedroom carpet age, months[††]		16	1.17 (1.01, 1.35)
Bedroom mattress age, 60 months			1.15 (0.92, 1.43)
Living room carpet age, 120 months		18	0.98 (0.86, 1.11)
Living room furniture age, months[††]			0.76 (0.61, 0.96)
Sample location, n (%)			
Bedroom carpet	44 (30)		Reference
Bedroom mattress	32 (22)		1.21 (0.67, 2.19)
Living room carpet	41 (28)		1.09 (0.63, 1.89)
Living room furniture	30 (20)		0.85 (0.47, 1.56)

Abbreviations: CI, confidence interval.

[†] 45 dust samples did not have enough dust in which to measure β-(1→3)-D-glucan concentration.

[‡] The detection limit was 0.0000625 μg/mg of dust.

[§] Estimated via linear regression models of the natural logarithm transformed values. Exponentiated regression coefficient and 95% CI is the geometric mean house dust β-(1→3)-D-glucan concentration ratio for a specified change in the independent variable or exp(β) – 1 = percent change in geometric mean house dust β-(1→3)-D-glucan concentration for a specified change in the independent variable.

[¶] Includes basement apartment (seven dust samples), condo (three dust samples), duplex (nine dust samples), four plex (three dust samples), four plex split level (two dust samples), split entry (five dust samples), trailer (two samples), tri-level split (four samples), and not applicable (six dust samples).

[††] Transformed by taking the natural logarithm of the original values.

Supplemental Table 2-18. Characteristics and house dust β-(1→3)-D-glucan surface load of low-income homes in Utah County, Utah, Summer 2017.

	House Dust β-(1→3)-D-glucan Surface Load[†]		
Characteristic	n (%)[‡]	Missing, n	Unadjusted exp(β)[§] (95% CI[§])
Home age, 10 years		4	1.12 (1.03, 1.22)
Home size, 50 m^2		4	1.24 (0.92, 1.67)
Style of home, n (%)			
Apartment	39 (27)		0.41 (0.21, 0.77)
Single story	24 (16)		1.89 (0.90, 3.96)
Two story	43 (29)		Reference
Other[¶]	41 (28)		0.86 (0.46, 1.62)
Residents, n			1.28 (1.12, 1.45)
Occupant density, residents/100 $m^{2††}$		4	1.58 (0.87, 2.86)
Bedroom carpet age, months[††]		16	1.18 (1.00, 1.41)
Bedroom mattress age, 60 months			1.22 (0.94, 1.58)
Living room carpet age, 120 months		18	0.94 (0.81, 1.10)
Living room furniture age, months[††]			0.74 (0.56, 0.97)
Sample location, n (%)			
Bedroom carpet	44 (30)		Reference
Bedroom mattress	32 (22)		1.24 (0.61, 2.51)
Living room carpet	41 (28)		1.13 (0.58, 2.20)
Living room furniture	30 (20)		0.78 (0.38, 1.61)

Abbreviations: CI, confidence interval.

[†] 45 dust samples did not have enough dust in which to measure β-(1→3)-D-glucan surface load.

[‡] The detection limit was 0.0000625 μg/mg of dust.

[§] Estimated via linear regression models of the natural logarithm transformed values. Exponentiated regression coefficient and 95% CI is the geometric mean house dust β-(1→3)-D-glucan surface load ratio for a specified change in the independent variable or exp(β) – 1 = percent change in geometric mean house dust β-(1→3)-D-glucan surface load for a specified change in the independent variable.

[¶] Includes basement apartment (seven dust samples), condo (three dust samples), duplex (nine dust samples), four plex (three dust samples), four plex split level (two dust samples), split entry (five dust samples), trailer (two samples), tri-level split (four samples), and not applicable (six dust samples).

[††] Transformed by taking the natural logarithm of the original values.

Supplemental Table 2-19. Spearman's rank correlation coefficients (p-values) for associations between house relative humidity, temperature, and dust mite allergen, endotoxin, and β-(1→3)-D-glucan concentration and surface loadof low-income homes in Utah County, Utah, Summer 2017.

	Weather or House Dust Analyte								
Weather or House Dust Analyte	72-hour mean relative humidity	72-hour mean temperature	Der f 1 concentration[†]	Der p 1 concentration[†]	Der f 1 or p 1 concentration[†]	Endotoxin concentration[‡]	Endotoxin surface load[‡]	β-(1→3)-D-glucan concentration[§]	β-(1→3)-D-glucan surface load[§]
72-hour mean relative humidity	1.00								
72-hour mean temperature	-0.04 (0.66)	1.00							
Der f 1 concentration	0.19 (0.02)[¶]	0.05 (0.53)[¶]	1.00						
Der p 1 concentration	0.09 (0.27)[¶]	-0.20 (0.02)[¶]	0.10 (0.21)[¶]	1.00					
Der f 1 or p 1 concentration	0.20 (0.02)[¶]	-0.07 (0.39)[¶]	0.77 (<0.0001)[¶]	0.64 (<0.0001)[¶]	1.00				
Endotoxin concentration	0.02 (0.86)[††]	-0.18 (0.04)[††]	0.09 (0.28)[¶,††]	0.17 (0.04)[¶,††]	0.18 (0.03)[¶,††]	1.00			
Endotoxin surface load	-0.03 (0.71)[††]	-0.20 (0.02)[††]	0.05 (0.58)[¶,††]	0.17 (0.04)[¶,††]	0.16 (0.05)[¶,††]	0.90 (<0.0001)[††]	1.00		
β-(1→3)-D-glucan concentration	-0.39 (<0.0001)	-0.32 (<0.0001)	-0.15 (0.07)[¶]	0.22 (0.01)[¶]	0.02 (0.80)[¶]	0.27 (0.002)[††]	0.23 (0.006)[††]	1.00	
β-(1→3)-D-glucan surface load	-0.33 (<0.0001)	-0.35 (<0.0001)	-0.11 (0.17)[¶]	0.22 (0.01)[¶]	0.05 (0.59)[¶]	0.30 (0.0004)[††]	0.30 (0.0003)[††]	0.93 (<0.0001)[††]	1.00

Abbreviations: Der f 1, *Dermatophagoides farinae*; Der p 1, *Dermatophagoides pteronyssinus*.

[†] 41 dust samples did not have enough dust in which to measure dust mite allergen concentration.

[‡] 45 dust samples did not have enough dust in which to measure endotoxin concentration or surface load.

[§] 45 dust samples did not have enough dust in which to measure β-(1→3)-D-glucan concentration or surface load.

[¶] Association with house dust mite allergen concentration above the detection limit compared to below the detection limit.

[††] Calculated from positive endotoxin concentration or surface load samples only

CHAPTER 3: Concluding Remarks and Future Directions

The work presented in this thesis summarizes research performed to understand exposure to dust mites, endotoxin and β-(1→3)-D-glucan in the home. Concluding remarks and future directions for health implications and fungal characterization and dust mite and endotoxin exposure will be addressed in individual sections.

3.1 Allergen exposure and asthma health implications

Dust mite exposure has been shown to be a potent inducer of asthma attacks and development of asthma itself. β-glucans appear to have significant potential to exacerbate symptoms of asthma, but more research on exposure must be done before we fully understand its potential and role in causing asthma attacks. Endotoxin can either be protective against development of asthma or act as a trigger in sensitized individuals depending on the timing and type of exposure. Evaporative coolers have already been shown to significantly influence levels of bioaerosols in the home, but the health implications of this are unclear. EC usage in the U.S. is heavily concentrated in the Rocky Mountain region [14,15]. Research throughout this region has produced conflicting evidence. A study done in Colorado showed no dust mite or mold sensitization due to evaporative coolers [19], while others in Reno Nevada showed increases in mold and fungal sensitization due to EC use [70,71]. More research on the topic must be done in order to fully understand the health implications associated with EC use.

Green & Healthy Homes Initiative Salt Lake (GHHI Salt Lake) Coalition consists of 23 agencies working together to help low-income families with a household member with asthma. GHHI Salt Lake provides approximately 60 households per year with services, including home assessments, interventions to reduce asthma triggers, and asthma education. GHHI will replace ECs with AC units in qualifying homes with asthmatic individuals. However, to date there is no

evaluation of the effectiveness of this intervention measure. We think it would be important to address this by sampling particulates and determining allergen levels before and after the change-out to determine if these asthma triggers diminish by replacing EC with AC.

Previous research comparing homes with evaporative coolers and central air have introduced a myriad of variables that are not possible to control for, such as humidity due to different cooking techniques between homes, number of adults versus children living in the different homes and home age and size. A study working with GHHI would eliminate most of these variables and present more consistent results since the comparisons between allergen levels in evaporative cooler versus central air would be done in the same homes. It would also be important to determine whether or not asthma improves for individuals living within the household before and after the change-out. This possible future research for this project would evaluate the effectiveness of the asthma intervention program, lead to better education and hopefully inclusion of health advisories with EC rebate programs as well as better health for asthmatic individuals.

3.2 Fungal characterization in homes with evaporative coolers

Research looking into β-glucan exposure and asthma is a relatively new topic. To date little research has been done to understand fungal exposure in homes with evaporative coolers. Research done in Colorado shows that evaporative cooler use does not lead to sensitization to mold and fungus [19]. Conversely, research done in Reno, Nevada, another semi-arid environment, shows that children in homes with evaporative coolers were significantly more likely to have positive skin tests to molds or mites [18]. More research must be done in order to better understand and clarify what is happening in these environments.

The results reported in this thesis with regards to β-glucans are novel in the field, and indicate that individuals in homes with ECs are exposed to higher levels of fungi than those in homes with AC. However, especially in the case of asthma, not all fungi have the same immunological effects. Different species of fungi are associated with allergic sensitization in humans while others are not. In order to determine whether or not homes in Utah with ECs expose individuals to higher levels of species of fungi associated with allergic sensitization, we propose doing an analysis and characterization of the microbial and fungal diversity in dust samples with PCR or high throughput sequencing. This future research can help determine if individuals living in homes with ECs in Utah county are at a higher risk for allergic sensitization and development of asthma due to higher exposure to these species of fungi.

Research on evaporative cooler use and endotoxin exposure has shown that sump water that is circulated throughout the system as the likely source of endotoxin [50]. Similar research done to determine the source of fungal exposure determined that it did not originate in sump water [71]. To better understand fungal exposure and its origin with evaporative coolers we propose comparing species of fungus from homes with evaporative coolers found indoors with those present in sump water and on the cooling pads. Determining the source of the fungal exposure would help individuals make more informed decisions about replacement of cooling pads during peak EC usage and subsequently lower levels of exposure to fungal species.

3.3 Endotoxin and dust mite exposure and asthma prevention

Dust mite allergen exposure is one of the most common asthma triggers, but an individual must be exposed to certain levels in order to cause an asthma attack [79]. We found that homes with ECs have greater odds of testing positive for dust mite allergens, however only two of our samples were above the clinically significant threshold of 2 μg/g of dust, meaning that

dust mite exposure due to evaporative cooler use is likely less of a concern than endotoxin and fungal exposures. Endotoxin exposure has been shown to be protective against asthma development, as well as an asthma trigger [43,44]. Studies have shown that early exposure to certain microbial profiles in the home, specifically ones associated with farm-like environments, can help prevent asthma development and allergic sensitization [26]. We propose early life exposure to this farm-associated microbiota through house dust as a possible avenue to be explored for asthma development prevention. Manipulation of at home exposures in populations genetically at risk for asthma development could potentially help train the immune system at an early age and avoid the hyper reactive response to allergens seen in individuals with asthma. This research and the future directions discussed in this chapter could help mitigate costs associated with asthma every year, lead to a better quality of life for asthmatic individuals and possibly help diminish the number of new cases of asthma diagnosed per year.

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APPENDIX: Genome Sequences of Nine *Erwinia amylovora* Bacteriophages

The following appendix contains a published genome announcement in Microbiology Resource Announcements.

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GENOME SEQUENCES

Genome Sequences of Nine *Erwinia amylovora* Bacteriophages

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ABSTRACT *Erwinia amylovora* is a plant pathogen belonging to the *Enterobacteriaceae* family, a family containing many plant and animal pathogens. Herein, we announce nine genome sequences of *E. amylovora* bacteriophages isolated from infected apple trees along the Wasatch Front in Utah.

At an estimated total number of 10^{31}, phages are by far the most abundant biological entity on the planet (1–7). They dramatically influence the evolution of bacteria by their ability to infect and kill their hosts and to transfer genetic material. *Erwinia amylovora* is a rod-shaped facultative anaerobic member of the *Enterobacteriaceae* bacterial family, which includes many well-characterized Gram-negative plant and animal pathogens, such as *Salmonella* spp., *Escherichia coli*, and *Klebsiella* spp. As the causative agent of fire blight, *Erwinia amylovora* infects members of the Rosaceae plant family, causing diseased areas to appear burnt (8–10). The isolation and characterization of phages that infect *E. amylovora* may aid in our understanding of these bacteria and provide potential treatment for this devastating agricultural disease. Herein, we announce the genome sequences of nine *E. amylovora* bacteriophages, vB_EamM_Aseslno, vB_EamM_Alexandra, vB_EamM_Bosolaphorus, vB_EamM_Desertfox, vB_EamM_MadMel, vB_EamM_Mortimer, vB_EamP_Pavtok, vB_EamM_SunLIRen, and vB_EamM_Wellington.

Phages were isolated from apple trees along the Wasatch Front in Utah that appeared to harbor fire blight infection. Phages were plaque purified through a minimum of three passages after amplification via enrichment culture (11). All nine phages reported in this announcement infect the *Erwinia amylovora* ATCC 29780 strain, as indicated by plaque assays, and their characteristics are summarized in Table 1. Genomic DNA was extracted (Phage DNA isolation kit; Norgen Biotek), a library was made using the Illumina TruSeq DNA Nano kit, and sample genomes were sequenced by Illumina HiSeq 2500 sequencing (250-bp paired end) and assembled with Geneious (12) version 8.1 using *de novo* assembly with medium-low sensitivity and various percentages of data. All phages circularized upon assembly and were annotated using DNA Master (http://cobamide2.bio.pitt.edu/computer.htm), giving preference for calls that gave full coding potential coverage.

The nine phages were grouped into five distinct clusters by genomic dot plot and average nucleotide identity analyses, as previously described (11), with the first three groups containing jumbo *Myoviridae*. The first jumbo group included four myoviruses, vB_EamM_Bosolaphorus, vB_EamM_Desertfox, vB_EamM_MadMel, and vB_EamM_Mortimer, which are similar to previously published *Erwinia* phage Ea35-70 (13), as well as other phages we have isolated (14). The second group included two jumbo myoviruses, vB_EamM_Aseslno and vB_EamM_Wellington, with similarity to the well-characterized *Salmonella* SPN3US phage (15) and related phages. The third is a single

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† Deceased. Charles J. Webb did not see or approve the final version of this paper.

TABLE 1 Properties of nine *Erwinia amylovora* bacteriophage genomes

Name	GenBank accession no.	SRA accession no.	Total no. of reads	No. of reads used	Assembly fold coverage (range [mean])	Length (bp)	No. of ORFs[a]	No. of tRNAs	G+C content (%)
vB_EamP_Pavtak	MH426726	SRX4597602	1,301,332	386,192	492–2,086 (1,069)	61,401	62	0	36.9
vB_EamM_SunLiRen	MH426725	SRX4597606	1,301,332	386,192	8,249–42,422 (13,566)	84,559	141	22	36.3
vB_EamM_Wellington	MH426724	SRX4597603	626,048	372,488	133–514 (329.7)	244,950	295	8	50.3
vB_EamM_Asesino	KX397364	SRX4597609	2,222,038	1,022,382	512–1,378 (1,037.7)	246,290	289	12	51.2
vB_EamM_Alexandra	MH248138	SRX4597608	381,540	200,005	63–516 (166.3)	266,532	349	0	49.8
vB_EamM_Bosolaphorus	MG655267	SRX4597604	778,168	326,344	83–555 (248.4)	272,228	321	1	49.4
vB_EamM_Desertfox	MG655268	SRX4597605	1,930,470	1,138,933	115–612 (352.9)	272,458	320	0	49.6
vB_EamM_Mortimer	MG655270	SRX4616109	2,581,160	287,396	47–207 (129.4)	273,914	325	1	49.5
vB_EamM_MadMel	MG655269	SRX4597607	1,604,720	1,443,568	567–1,577 (1,213.9)	275,000	321	0	49.4

[a]ORFs, open reading frames based on current annotation.

jumbo myovirus, EamM_Alexandra, which has similarity to previously published *Erwinia* phages EamM_Yoloswag (14) and EamM_Y3 (16). Podovirus vB_EamP_Pavtok and myovirus vB_EamM_SunLiRen are similar to *Erwinia* phages PEp14 and phiEa21-4 (17), respectively. The three jumbo myovirus groups package DNA by headful packaging (14) based on homology to phage phiKZ terminase (18), and their bp 1 was chosen by alignment to their phage family. PhageTerm (19) was used to determine the packaging strategy of SunLiRen and Pavtok. SunLiRen appeared to have headful packaging, and its bp 1 was assigned based on homology alignment to *Erwinia* phage phiEa21-4, while the packaging strategy of Pavtok is unknown, and its bp 1 was assigned due to homology to PEp14.

Data availability. The GenBank and SRA accession numbers for the nine *Erwinia* bacteriophages are listed in Table 1.

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