Assessment of Pre- and Post-Hurricane Katrina New Orleans Mold

Exposures and Biomarkers of Exposure

A Dissertation Submitted

To the Department of Global Environmental Health Sciences

Tulane University School of Public Health & Tropical Medicine

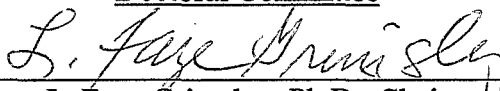In Partial Fulfillment of the Requirement for the Degree of Doctor of

Philosophy

By

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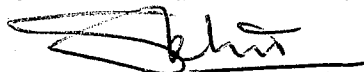November 17th, 2011

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I. Abstract

Background: There is a potential for mold exposure in water-damaged New Orleans homes after Hurricane Katrina.

Objectives: Overall purpose: To determine if microbial concentrations and composition of air and dust in New Orleans households were different in pre-Katrina and post-Katrina environments. Secondary: 1) To characterize airborne mold exposure pre-Katrina from comparing two mold spore sampling impactors 2) to establish if significant correlations existed between fungal and bacterial markers in dust from previously water-damaged New Orleans homes and characteristics of the homes, and 3) to determine if total and specific Immunoglobulin E (IgE) antibodies positively correlated with indoor mold exposure duration and severity in New Orleans homeowners and workers.

Methods: Pre-Katrina, 47 total indoor and 9 outdoor air samples were collected in asthmatic patients' homes. Post-Katrina, the total numbers of samples collected were: 30 dust, 41 indoor air, 13 outdoor air and 20 blood samples. Pre- and post-Katrina health data were collected using questionnaires.

Results and Conclusions: Basidiospores, ascospores and *Penicillium/Aspergillus* were predominant pre- and post-Katrina. Non-renovated homes had higher microbial marker levels. Of the twenty blood samples, eight sera had elevated total IgE, primarily for control and renovated homeowners with low indoor/outdoor mold spore counts. Mold antibody levels did not correlate with mold exposure duration and severity. Overall, Indoor/Outdoor non-viable spore and viable colony mold ratios pre-Katrina were 0.19 and 4.16, respectively. Overall I/O non-viable post-Katrina mold spore ratio was 2.74, indicating a higher average non-viable spore count post-Katrina than pre-Katrina.

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II. Background and Significance

After Hurricane Katrina first landed in Louisiana on August 29th, 2005, more than 100,000 New Orleans households, approximately 48% of the total number of residences in the Greater New Orleans area, were inundated with 2 feet or more of floodwater (HUD 2005). In some of the hardest hit districts, i.e. the Lower Ninth Ward, Gentilly, New Orleans East, Lakeview, and Mid-City, 42% to 87% of the homes had flood depths exceeding 2 feet. From landfall until the completion of the dewatering process on Oct. 11th, many of these buildings festered in pools of stagnant water, ultimately resulting in large swaths of mold-infested buildings (Roper et al 2006). The nutrient-rich organic matter and high moisture levels within the buildings' walls, furnishings and other water-logged materials were prime breeding grounds for indoor fungi (HUD 2006); these fungi were already ubiquitous in outdoor areas of New Orleans, especially due to the relatively high temperatures and humidity throughout the area.

In 2006, the Centers for Disease Control (CDC) determined that 46% of the 112 New Orleans area homes surveyed exhibited visible mold; 17% had extensive mold growth which covered more than half of a wall in the home (CDC 2006). Of the homes, 67% also had significant roof damage, leading to additional moisture intrusion. Of the 235 total residents and remediation workers interviewed, 69% and 35%, respectively, failed to consistently wear respiratory protection while cleaning up moldy homes. These findings indicate that environmental conditions to which the residents and remediation workers are returning may result in increased exposure to microbial markers.

Although there are no federal standards regulating inhalable mold exposure, the development and exacerbation of asthma and respiratory allergies have been frequently associated with dampness, indoor fungal growth and other biological agents (Gaffin et al 2009; Jacob et al 2002; Mendell et al 2011). These microbes may cause Type I or Immediate hypersensitivity to respiratory allergens, which activates Immunoglobulin Type E (IgE) antibodies. IgE antibodies, produced by plasma cells in the skin, lungs and mucosal membranes, are often generated in response to fungal allergens, dander, pollen and other antigens (Janeway et al 2001). Unlike the other 4 antibody isotopes, G, A, M and D, IgE is strongly bound to tissue mast cells, basophils and activated eosinophils. In a person allergic to mold, a cross-reaction of mold-specific antigens and IgE leads to an allergic response within the affected individual.

Approximately 10% to 60% of people who are exposed to mold and are susceptible due to genetic factors (atopic) may develop mold allergy, as indicated by skin tests (IOM 1993). The actual percentage of atopic people is unknown; however, it is commonly accepted as 20 to 30% of individuals or up to 6% of the general population (Horner et al 1995; Rabito et al 2010). However, the World Health Organization (WHO) concluded that other household allergens, such as dust mites, pet dander and cockroaches, may be cofactors contributing to the reported health effects (WHO, 2009).

Decades ago, pioneering studies were conducted by researchers, such as those by Dr. John Salvaggio. An investigation was launched after an apparent 'epidemic' of increased hospitalization rates in atopic or allergic New Orleans residents and a potential increase

in the exacerbation of asthma during peak warm seasons (Salvaggio et al 1973; Horner et al 1991). It was found that the increased asthma attacks resulted from elevated exposure to 'natural' particulates, such as basidiospores and other fungal antigens, dust mites, house dust and human dander. Also, the serum antibodies for these allergens were elevated, even in non-atopic individuals; this suggested that New Orleans residents may have higher background levels of antibodies to these factors from constant exposure.

Displaced residents returning to their water-damaged properties, and the influx of volunteers, laborers and 'do-it-yourself' homeowners cleaning up the mold-contaminated habitats, face potential respiratory, skin and eye infections from exposure (Metts 2008; NYCDOH 2002). Reported respiratory symptoms positively correlated with exposure to flooded homes, and negatively correlated with respirator use (Cummings 2008).

This research study focused on assessing pre- and post-Hurricane Katrina mold exposures from three aspects by: 1) characterizing airborne mold exposure pre-Katrina from comparing two mold spore sampling impactors, 2) analyzing dust and air samples recovered from post-Katrina New Orleans homes for fungal and bacterial markers, and 3) determining residential environmental markers and mold antibody levels for control and water-damaged homeowners, volunteers and laborers.

Pre-Katrina, two bioaerosol sampling impactors were field-tested in New Orleans' homes with at least one doctor-diagnosed asthmatic resident, and a health-related questionnaire was completed by each participant. Post-Katrina, dust samples collected from homes in

various stages of repair in New Orleans were analyzed for mycotoxins, endotoxins, ergosterol (Erg) and muramic acid (MA), using gas and liquid chromatography-tandem mass spectrometry (GC-MSMS and HPLC-MSMS, respectively). Ten dust samples were analyzed with Enzyme-linked Immunosorbent assay (ELISA) for *Aspergillus versicolor* and *Stachybotrys chartarum*. This was only the second post-Hurricane Katrina research to measure New Orleans household dust for these markers. Air and dust samples for select homes were also analyzed for (1-> 3)-β-D-glucans and endotoxins (bioactivity-LAL) by the University of Cincinnati (U.C.) as part of a larger HUD-funded study; air samples from 3 of the homes were analyzed by U.C.for non-viable fungal spores. Health questionnaire data for owners of the water-damaged homes were also obtained.

Another post-Katrina objective was to determine if total and mold-specific IgE antibody levels positively correlated with mold exposure duration and severity of indoor mold spore counts among New Orleans homeowners, volunteers and laborers. It was assumed that elevated levels of indoor airborne mold spores would elicit measurable mold antibody responses in the non-control participants' bloodstreams, and the second assumption was that the molds present four years after Hurricane Katrina were primarily dead, which would still be allergenic. To the authors' knowledge, ours was the first post-Hurricane Katrina New Orleans' research which examined potential mold exposure for remediation workers and homeowners using questionnaires, IgE levels in blood serum samples and environmental air and dust samples. It was also the first research since the 1970's to analyze blood sera of New Orleans residents for multiple mold biomarkers after inhalation exposure (Salvaggio et al 1973). Additionally, we provide the only non-

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internal research data to use the BioCassette for viable mold spore collection and comparison with single stage Andersen N6 impactor.

Overall, this research provides more insight into the environmental impact of natural disasters and subsequent flooding, which encourages mold growth and the propagation of biological by-products. The health effects of such disasters may have far-reaching, long-term consequences on the quality of life of those who are exposed to biological agents.

III. Literature Review

Airborne mold spore sampling

Different types of air sampling devices are available for conducting environmental assessments for mold and other bioaerosols, which fall into the following general categories: 1) impactors, which rely on inertia to drive particulates onto sampling media; 2) impingers, which use diffusion to collect particulates in liquid; and 3) filtration, which use both inertia and diffusion to collect matter onto filters (Riemenschneider et al n.d.). However, only impactors will be discussed in this section, as three of the air sampling methods used in this research (single stage Andersen N6, BioCassette and Air-O-Cell cassettes) all fit into the aforementioned category. The sole exception is the Button aerosol sampler, a filtering device, which was used in the HUD study.

Viable airborne mold spore collection efficiencies of impaction samplers, i.e., Andersen sampler (stages 1 - 6), Air-O-Cell cassettes, BioStage, Burkard portable, Reuter Centrifugal Sampler (RCS) and Surface Air System (SAS), have often been compared by researchers. However, external factors, i.e. wind speed at the sampling site, temperature, relative humidity and particle size distribution, may affect sampler performance (Adhikari et al 2003; Yao and Mainelis 2007). Andersen, Burkard and BioStage have similar operating principles, as air is drawn through multiple orifices at 28.3 L/min. (Mehta et al 1996). However, the RCS, SAS, Air-O-Cell cassettes and Button samplers have different operating mechanisms. The RCS, at a flow rate of 50 L/min., deposits particulate matter onto media contained in plastic strips with 1 cm^2 wells. The SAS 90

draws air at 90 L/min. through +200 orifices, depositing airborne matter onto RODAC contact plates.

Some samplers, i.e., Air-O-Cell cassettes and button aerosol samplers, are capable of collecting mold spores and other particulates for either viable or non-viable mold analysis. Air-O-Cell cassettes use inertial impaction to direct airborne mold spores and particulate matter, at 15 L/min., through a slit onto adhesive-coated glass slides (Zefon 2011). Button aerosol samplers draw air in, at 4 L/min., through a curved stainless steel inlet with multiple orifices to deposit matter onto a 25 mm filter; filters may consist of either gelatin, polyvinyl chloride (polycarbonate), glass fiber or mixed cellulose ester (Adhikari et al 2003; Aizenberg et al 2000; SKC 2010; Yao and Mainelis 2007). The d_{50}, the aerodynamic diameter at which at least 50% of the particles are collected, for the impactors are: Andersen (single stage) - 8.0 µm, Air-O-Cell- 2.3 µm, BioStage- 0.6 µm, Burkard- 2.56 mm, Button sampler- 2.3 µm, RCS- 6 mm and SAS 90- range of 2.0 to 4.0 µm (Aizenberg et al 2000; Mehta et al 1996; Trakumas et al 1998).

When Air-O-Cell cassettes, Burkard personal volumetric sampler and button aerosol sampler were tested for total mold spore collection, the button sampler was more efficient at collecting smaller spores, i.e. from *Cladosporium* than *Penicillium* (Aizenberg et al 2000). In one study of viable mold spore collection, SAS continually recovered fewer colony forming units than the Andersen single stage N6 impactor (Bellin and Schillinger 2001). RCS and SAS each collected 80-90% of the mold spores in another study, with BioStage collecting only 60% (Yao and Mainelis 2007). In a comparison of four air

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sampling impactors, RCS, Andersen, SAS 90 and Air-O-Cell, total mold spore collection efficiencies (Pearson: $r = 0.60 - 0.85$; $p < 0.001$) were consistent in non-water-damaged Canadian buildings (Lee et al 2004). Andersen 2-stage and Burkard recovered comparable levels of fungi, whereas RCS Plus and SAS Super 90 collected considerably lower levels than the other two devices (Mehta et al 1996). RCS was more efficient at overall mold spore count recoveries compared to single stage N6 Andersen, especially up to an optimum sampling time of 6 minutes (Saldanha et al 2008). However, the RCS was less efficient than the six-stage Andersen at qualitative mold spore recovery (Tavora et al 2003).

In 2003, the BioCassette was introduced by Environmental Lab (EMLab) as a more efficient alternative to the "gold standard" sampler, single stage Andersen N6 impactor, for viable mold sampling (EMLab 2010). Both devices use the same operating principles: air is drawn at 28.3 L/min through multiple orifices to deposit spores and particulates on Malt Extract Agar, MEA (Figures 1 and 2). However, the single stage Andersen N6 impactor consists of three basic components: an inlet cone made of aluminum, a jet stage and base plate, all of which is secured with three spring-loaded clamps and o-ring gaskets (Thermo Scientific 2009). The BioCassette, a single-use device pre-filled with MEA for mold spore sampling, touted the following advantages: 1) no field-cleaning required and no risk of cross contamination, and 2) compact, lightweight and more cost-effective than Andersen (EMLab 2010).

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Figures 1 and 2. BioCassette^{TM} and Single Stage N6 Andersen Impactor
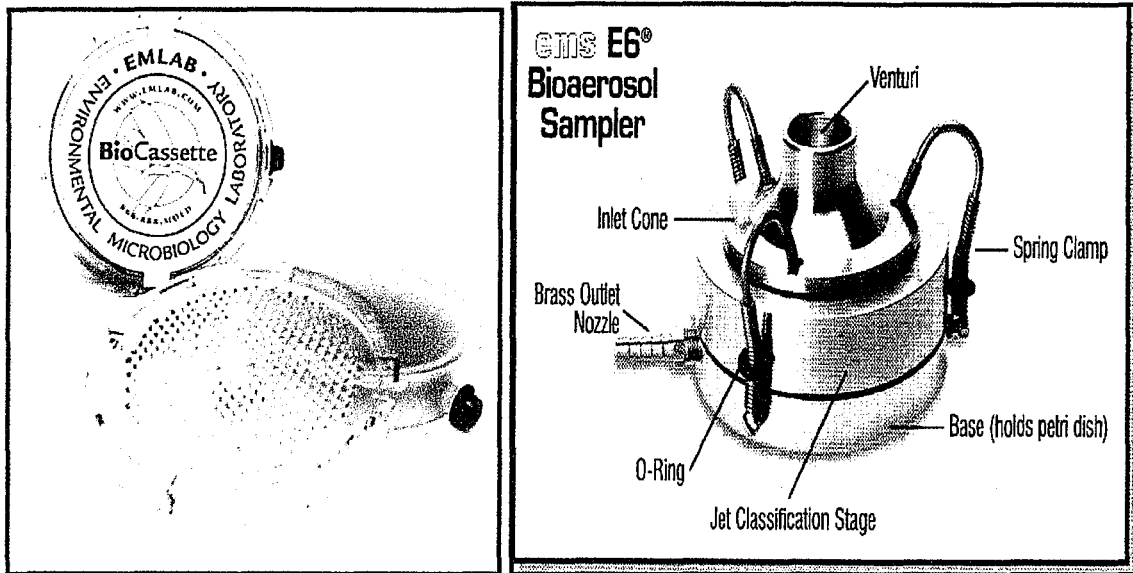
Sources: Photos of BioCassette and Andersen reprinted from EMLab.com and

BioIdea.net, respectively

EMLab conducted field testing of the two devices in Northern California by

simultaneously operating the samplers side-by-side, and fifty pairs of samples were

collected from five locations (Purves and Georgianna 2003). The BioCassette

consistently produced at least 0.5 colony forming units per cubic meter (CFU/m^3) more

than the Andersen samples. Mann Whitney testing yielded no statistically significant

difference between the samplers (p = 0.20).

Fungal Taxonomy and General Guidelines

Flooding, both coastal and inland, primarily occurs when soil in an area becomes

saturated due to at least one of several meteorological conditions: 1) localized prolonged

heavy rainfalls, 2) rapidly melting snow following a severe snowstorm or 3) high wind

activity from a hurricane, cyclone or tsunami pushes bodies of water inland (NOAA 2011). The saturated soil or closest water source becomes unable to accommodate the excess water, which then inundates nearby communities. The highest recorded flooding in the Mid-Western region of the United States in 131 years occurred in 1993, due to melting snow from record storms, followed by prolonged heavy rainfalls (USGS 2010). Similar conditions, from Jan. to Sept. 2008, contributed to near record flooding in the Mid-West, primarily affecting Arkansas, Illinois, Indiana and Missouri. Regardless of the region or cause of flooding, studies from prior events worldwide have found significant associations between moisture intrusion and potentially increased household microbial exposure.

In post-Hurricane Katrina New Orleans studies, the following mold genera were commonly found in water-damaged buildings: *Aspergillus* and *Penicillium* species, *Cladosporium, Paecilomyces, Trichoderma, Zygomycetes* and *Stachybotrys* (Chew et al 2006; Rao et al 2007; Solomon et al 2006). Viable mold spore concentrations in water-damaged homes ranged from 22,000 to 515,000 CFU/m^3, and non-viable mold spore counts ranged from 11,000 to 645,000 spores/m^3 over a 24-hour collection period (Chew et al 2006; Solomon et al 2006). Indoor/outdoor total airborne fungal spore ratios in 14 Taiwanese households averaged 0.6 before Typhoon Morakot and 1.4 after the storm; flooded rooms had average culturable mold and total fungal spore counts of 13,440 CFU/m^3 and 201, 582 spores/m^3, respectively, with increased levels of *Aspergillus* species (Hsu et al 2011). Water-damaged residences of asthmatic children in Cleveland

had significantly higher levels of *Scopulariopsis brevicaulis* and *Trichoderma viride*, a water indicator mold, than homes without water damage (Vesper et al 2006).

In some geographical locations, leaf surface fungi, i.e., *Alternaria* and *Cladosporium*, primarily contribute to the mold spore burden in indoor and outdoor environments; more soil fungi, such as *Aspergillus*, *Penicillium* and *Stachybotrys*, are typically prevalent in homes with moisture intrusion (Levetin and Dorsey 2006). Non-water-damaged homes in southeastern regions may exhibit characteristics similar to those in Dr. Horner et al's study of 50 Atlanta-based homes, such as predominantly finding leaf surface fungi indoors, as opposed to soil fungi (2004). Fungi commonly associated with water intrusion were not found in the samples; dust samples with fewer than twenty percent of the colonies comprising leaf surface fungi were less likely to be from non-water-damaged homes. Another study examined the viable fungal content in 12, 026 air samples throughout the United States from 1996 to 1998; the most common fungi found in all regions, regardless of season, were: *Cladosporium*, *Penicillium*, non-sporulating fungi and *Aspergillus* (Shelton et al 2002). The highest mold colony levels were observed in areas with warmer climates, i.e., in the Southwest, Far West and Southeast regions, primarily in the summer and the fall. Indoor colony counts ranged from below the detection limit of 12 CFU/m^3 to more than 10,000 CFU/m^3. *Cladosporium* and *Penicillium/Aspergillus* are among the most notable in causing immunological reactions, such as IgE production and pulmonary inflammation (Shen et al 2007).

Outdoor spore trap data 1 year post-Katrina (PK) were compared with outdoor spore trap

data from the Head-off Environmental Asthma in Louisiana (HEAL) study more than 2

years post-Katrina (Grimsley et al 2010). PK samples had significantly higher levels of

mold spore concentrations and types commonly associated with water-damage and soil

compost, although there were almost four times as many HEAL samples. HEAL samples

also reflected the seasonal variations in outdoor mold spore concentrations, unlike PK

samples.

Recently, the United States Environmental Protection Agency (EPA) and the Department

of Housing and Urban Development collaborated to devise a national system, known as

Environmental Relative Moldiness Index (ERMI), for characterizing indoor mold

contamination (Vesper et al 2007). The system uses a database of DNA primers in mold-

specific quantitative polymerase chain reaction (MSQPCR) to detect at least 36 mold

species in indoor household dust samples (Figure 3) (EPA 2010). It was designed to

estimate the potential fungal burden in the home and measure the likelihood that the mold

exposure may elicit symptoms in sensitive residents. The species identified include

water-indicator molds as well as soil and leaf surface molds, and the system places high

mold-burdened homes in a higher quartile than homes with lower ERMI levels (Vesper et

al 2007; Vesper et al 2011). However, the method is only a general guideline for

interpretation of biological indoor air quality.

Figure 3. ERMI diagram- National data
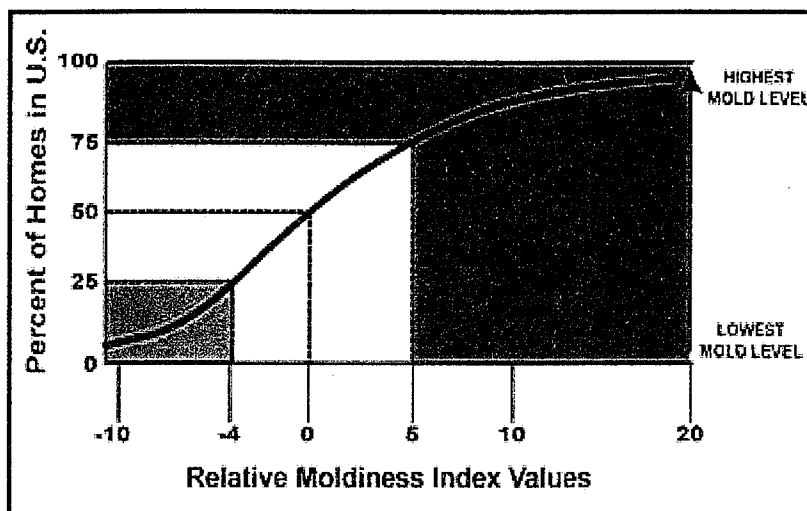
Sources: EMLab P&K 2010; EPA 2010

Despite the lack of federal regulations regarding inhalation mold exposure, there are

some general guidelines cited by the Natural Resources Defense Council (NRDC) for

"clean" vs. "moldy" home spore counts (Table 1) (NRDC 2005). Although no home can

ever be completely free of mold spores, a "clean" home according to Baxter et al was

defined as no evidence of mold growth, no history of flooding and no signs of moisture

intrusion; a "moldy" home was determined to be one with any visible mold growth,

especially if the patch was larger than 1 ft.^{2} (2005). NRDC and the National Allergy

Bureau suggested that outdoor mold spore counts in New Orleans exceeding 50,000

spores/m^3 were very high, with low counts ranging from 1-6,499 spores detected (NRDC

2005). Generally 'acceptable' indoor viable mold colony counts were determined by the

American Conference of Governmental Industrial Hygienists (ACGIH) as levels between

100 and 1,000 CFU/m^3 (Jo et al 2005).

Table 1. General Guidelines for Indoor Mold Levels

'Clean' vs. 'Moldy' Indoor Air Guidelines (spores/m³)	
Total Spores	Clean ≤ 1,200
	Moldy ≥ 1,300
Penicillium/Aspergillus	Clean ≤ 750
	Moldy ≥ 900
Ascospores/Basidiospores	Clean ≤ 1,200
	Moldy ≥ 1,300

Sources: NRDC 2005; Baxter et al 2005

Other organizations have also provided guidelines related to mold and indoor air quality.

The World Health Organization (WHO) maintains that indoor dampness is the culprit in

increasing microbial growth and contributing to subsequent exposure in building

occupants (WHO 2009). Structures in low-income areas with inadequate ventilation and

overcrowding were at higher risks of developing mold-related issues, accounting for 75%

of the 110 homes observed in refugee camps in Gaza. It has been estimated that at least

20% of the buildings worldwide have at least one sign of dampness, such as leaks,

condensation on indoor surfaces, water marks and discoloration on building materials,

visible mold growth and/or moldy odors (IOM 2004). The New York Health Department

provides general recommendations for mold assessment and remediation, such as

correcting the cause of the moisture source, removing visible mold with gentle cleansers,

discarding porous, moldy clothing and belongings and maintaining humidity levels below

65% (NYDOH 2008).

Microbial markers in households and potential health effects

Fungal fragments, mold spores, mycotoxins, microbial volatile organic compounds and bacterial components may become aerosolized and inhaled and/or ingested when dust and building materials are disturbed (Adhikari et al 2009; Bloom et al 2009a; Butte et al 2002). Exposure to these microbial markers and other environmental factors, such as dust mites, cockroaches and pet dander, may contribute to negative health effects generally categorized as "sick building syndrome" (Saijo et al 2011; Sauni et al 2011; Straus 2011). Endotoxins may cause respiratory irritation, and are commonly found indoors (Adhikari et al 2009). However, there are currently no exposure standards for inhalation of molds, mycotoxins or endotoxins (Robbins et al 2000; Poole et al 2010).

Some reported health effects from exposure to fungal and bacterial markers include: upper and lower respiratory infections (i.e. affecting the nasal and throat regions in the former and the pulmonary bronchial and alveolar tubes in the latter), allergic rhinitis, wheezing, coughing, dyspnea, bronchitis, exacerbation of asthma and directly causing other respiratory diseases, such as Aspergillosis (Fisk et al 2010; HUD 2006; Klich 2009; Mendell et al 2011; Sahakian et al 2008). Susceptible subpopulations, such as children, the elderly and other immunocomprised individuals, are at higher risk of developing asthma or opportunistic mycoses (Arshad 2010; IOM 2004; Roberts and Dickey 1995).

An estimated 20 million people in the United States currently have asthma, resulting in more than $18 billion in estimated healthcare costs and lost productivity (AAFA 2006). As of 2002, the number of adults with self-reported asthma exceeded 15 million cases

(Denning et al 2006). In Chicago from 1985 to 1989, the odds of death, among children and young adults, potentially due to mold-induced asthma was more than twice as high when outdoor mold levels exceeded 1000 spores/m^3 than from elevated pollen levels (Targonski et al 1995). African American young adults in Chicago from 1968 to 1991, Home characteristics, i.e. the presence of pets, visible mold and/or water damage, home in disrepair and household cleaning habits, were all associated with microbial levels and increased new onset asthma or exacerbation of pre-existing asthma in children (Hyvärinen et al 2006; Maier et al 2010). In a New York study, rates of asthma in adults and children strongly correlated with the presence of mold in the homes, even more so than with pets, cockroaches or tobacco smoke (Nguyen et al 2010). Low-income asthmatic inner-city children in the U.S. potentially face other in-home respiratory irritants in addition to fungi, such as cockroaches, rodents, and indoor furred or feathered pets (Eggleston et al 1998; Gruchalla et al 2005; O'Connor et al 2004).

Spirometry is commonly used to measure the respiratory health and reduced forced expiratory volume (FEV) of those with suspected or confirmed mold sensitization, allergies or disease (Norbäck et al 2011). The American Lung Association of Louisiana found that out of the 1,600 New Orleans residents checked for impaired respiratory health by August 2006, "about 25% had mild to moderate reduced lung function." (Szabo 2006). A Tulane Health Sciences Center pulmonologist reported seeing new patients who developed sustained coughing after a Katrina (dubbed the "Katrina cough" by the local media), and others who experienced an exacerbation of pre-existing respiratory ailments. However, it was unknown if the impaired lung function or respiratory symptoms could be

directly attributed to increased microbial exposures due to the hurricane. Chronic exposure to airborne endotoxin levels exceeding 50 EU/m^3 has been potentially associated with impaired lung function, although lung function naturally declines approximately 20 to 30 mL per year due to aging (Rando, Guest Lecture).

Strong correlations (r = 0.88; p < 0.001) have been observed between levels of muramic acid, ergosterol and 3-OH fatty acids and concentrations of culturable fungi and biological endotoxin activity in household dust (Saraf et al 1997). Airborne endotoxin levels in post-Katrina New Orleans' flooded household studies ranged from 7.0 x 10^2 to 9.3 x 10^4 EU/m^3, 22.85+ 60.95 EU/m^3 to 36.91 + 50.94 EU/m^3, 22.3 EU/m^3 (geometric mean), 40.2 EU/m^3 (geometric mean), and 0.6 to 8.3 EU/m^3 (Adhikari et al 2009; Adhikari et al 2010; Chew et al 2006; Rao et al 2007; Riggs et al 2008; Solomon et al 2006). Personal endotoxin exposures in school-aged asthmatic children correlated better with ambient air endotoxin levels than with indoor or outdoor residential levels, indicating potential microbial exposures during daily activities even while away from home (Delfino et al 2011).

Gram-positive bacteria are represented in research by the presence of N-acetyl muramic acid (NAM or MA), which comprise murein peptidoglycan found exclusively in both gram-negative and gram-positive cell walls (Todar 2011). MA and N-acetyl glucosamine (NAM) form glycosidic crosslinks with techoic acids to form the mesh-like structure of gram-positive bacteria cell walls, which are at least 5 times thicker than gram-negative peptidoglycan layers. Actinomycetes, thermophilic gram-positive bacteria, secrete

exotoxins, which have been associated with higher carbon chain lengths, i.e. C:16 and greater (Sebastian et al 2005).They may also contribute to the development of hypersensitivity pneumonitis, a lung condition caused by inhalation of myriad organic particles, and new onset asthma in exposed children (Hyvärinen et al 2006; Navarro et al 2006; Thrasher and Crawley 2009).

From 1993 until 2000, at least 30 children in Cleveland, potentially exposed to Stachybotrys in homes with significant water damage, presented with cases of idiopathic pulmonary hemosiderosis/hemorrhaging (IPH), with five fatalities (CDC 2000; Dearborn et al 1999; Dearborn et al 2002). These results led another researcher to examine the cases of pulmonary hemosiderosis and potential fungal associations in deceased North Carolina infants and from 1978 to 1996 (Jackson and Gilliland 2000). Only one case of pulmonary hemosiderosis was present, with no fungal contamination evident in tissue samples; the remaining deaths were attributable to Sudden Infant Death Syndrome (SIDS). Stachylysin, a metabolite produced by *Stachybotrys*, was isolated in dust samples from seven of the Cleveland infants' homes and from the lungs of a infant in Texas, potentially implicating *Stachybotrys* and its metabolites in the development of IPH (Vesper and Vesper 2002).

Most fungi have the potential to release non-volatile mycotoxins, which may cause allergic reactions in humans (Neville and Kurtz 2009). Respiratory and eye irritation may also be caused by microbial volatile organic compounds (mvocs) produced by some fungi and bacteria, usually in warm and humid enclosed spaces. The mvocs, low molecular-

weight gaseous chemical compounds released into the air, may account for the "musty" odor often associated with moldy environments (Korpi et al 2009; Polizzi et al 2009). Not all mold species release mycotoxins or mvocs at all times. Environmental conditions appear to influence the frequency, amount and duration of mycotoxin and mvoc production, such as warm room temperature, type of substrate and lighting (Kilambi 2006; Korpi et al 2009). For example, some *Cladosporium* species need periods of light to enhance spore and mycotoxin production, and *Fusarium proliferatum* exhibited accelerated growth and fumonisin production by 40% after exposure to light (EMLab 2010; Fanelli et al 2011).

Aspergillus species, especially *Aspergillus flavus*, are often the culprits in the production of aflatoxins and gliotoxin (Klich 2007; Sullivan et al 2001). Sterigmatocystin, an aflatoxin precursor, is primarily produced by Aspergillus versicolor (Cai et al 2011). Aflatoxin B1, which has been the most researched strain compared to B2, G1 and G2, has been designated by the International Agency for Research on Cancer as a Group I human carcinogen (Bennett et al 2003; IARC 1993; Miliţă et al 2010). Inhalation of AFLAB1 has been associated with lung and liver cancer in some cases of human exposure (EPA 2010).

Non-sporulating fungi, also known "sterile mycelia", cultured on media may potentially induce respiratory symptoms in some exposed persons, as spores may still be produced in natural environments (Kilambi 2006). Most mycotoxin data to-date focused on ingestion and dermal exposure (Brandt et al 2006; Paterson et al 2010; Sullivan et al 2001).

Primary food and feed mycotoxin contaminants are not commonly found in the indoor

air; however, some of their properties may prove useful in understanding the

complexities involved with associating indoor mold and mycotoxins with potential health

effects (Jarvis et al 2005).

Even in buildings with adequate ventilation and no visible mold growth, microbial

volatile organic compound (mvoc) detection and negative health effects in children have

still been observed. In 8 primary schools in Sweden, levels of mvocs averaged 423 ng/m^3

indoors and 123 ng/m^3 outdoors; viable mold levels were 360 and 980 CFU/m^3,

respectively (Kim et al 2007). Difficulty breathing at night and doctor-diagnosed asthma

were associated with higher levels of mvocs. Children who were exposed to mold were

twice as likely to report moodiness as those who were exposed to terbutaline (medication

used to treat asthma and other lung complications) or did not have any known chemical

exposures (Kilburn et al 2009). Six of the 35 children with known mold/mycotoxin

exposure also had a clinical diagnosis of autism spectrum disorder; they had an average

of 12.2 abnormalities, consisting primarily of balance, vision and slow blinking reflex

issues.

The mechanism by which fungi generate and release their by-products, and the health

effects which may result in exposed populations, requires further study. One recent

mycotoxin study exposed mice with acute- and chronic-induced asthma intranasally to

gliotoxin (primarily produced by *Aspergillus* species) and patulin, which increased the

allergic immune response in their lungs and bone marrow from oxidative stress (Schutze

et al 2010). When the mice were treated with an antioxidant, specifically N-acetylcysteine intraperitoneally prior to mycotoxin exposure, their lungs did not become inflamed. This important finding may prove promising to humans exposed to mycotoxins due to inadequate indoor air quality.

Biomarkers of mold exposure and analytical detection methods

At the present time, there are no 'gold standard' biomarkers for human exposure to indoor and ambient mold. However, antibodies generated in response to and spores from molds and mycotoxins have been isolated from tissue samples, blood sera and saliva in sensitive, exposed populations. Analytical methods used, often in conjunction, to detect biomarkers (typically IgE) from fungal sensitization and allergies include: Enzyme-linked Immunosorbent (ELISA) assay, Fluoroenzymeimmunoassay (ImmunoCAP), skin prick testing (SPT) and anterior rhinomanometry (nasal inhalation challenge) and radioallergosorbent (RAST) assay (Helbling et al 1998; Phadia 2010). In vitro tests, ELISA, ImmunoCAP and RAST, produce comparable results, given that the primary difference between the methods is the type of enzyme attached to the capture anti-IgE antibody during analysis; β-galactosidase is typically used with ELISA and ImmunoCAP, whereas RAST uses isotope I^{125} (Crameri et al 1996; Nordvall et al 1990; Nüsslein et al 1987). ImmunoCAP has been instrumental in measuring IgG and IgE to molds, which may be useful in distinguishing allergic bronchopulmonary aspergillosis (ABPA) from *Aspergillus fumigatus* sensitization (Hemmann et al 1999; Okuneva et al 2010; Sarma et al 2003). Total serum IgE measured with ELISA, ImmunoCAP and RAST also correlated with positive SPT results (Mari et al 2003; O'Driscoll et al 2009; Sharma et al 2011).

However, SPT alone may not be as conclusive as in vitro tests for confirming mold allergies (VanArsdel and Larson 1989).

The most common in vivo allergy test, skin prick testing, involves an allergist placing extract from the suspected allergens on the patient's arm and pricking the area with a needle to introduce the antigen subdermally; an allergic reaction is indicated by the presence of a red 'wheal' or raised bump at the site (AAAAI 2011). In a post-Katrina study of mold exposure and New Orleans' residents, only 10% of those tested had a positive skin test to any mold; no associations were evident between the positive skin test results and the self-reported exposures to mold and indoor dampness (Rabito et al 2010). Young adults in suburban and rural areas in India were 1.5 to 2.7 times as likely to be sensitized to fungi in skin prick testing than their urban peers (Mahesh et al 2010). Skin reactivity tests often produced reactions to basidiomycetes and/or ascomycetes in residents in warmer regions (Lehrer et al 1986; Lehrer et al 1994; Rivera-Mariani et al 2011a; Rivera-Mariani et al 2011b). When basidiomycetes elicited a positive skin test in only 4% of participants in a study, anterior rhinomanometry caused reduced nasal breathing capacities in 73% of sensitized individuals (Helbling et al 1998).

Gliotoxin, an immunosuppressive mycotoxin produced by several *Aspergillus* species (especially *A. fumigatus*), was isolated in lung and sera samples from cancer patients diagnosed with Aspergillosis (Thrasher and Crawley 2009). Residents of water-damaged buildings had significantly higher levels of mold-specific antibodies (particularly IgA, IgM and IgG) in blood sera analyzed with ELISA than those in non-water-damaged

homes; subjects in the former category also exhibited increased impairment of sensory and motor nerves (Campbell et al 2002; Vojdani 2003a; Vojdani et al 2003c). Single and repeated exposures to inhaled mold spores by various species, especially from *Aspergillus*, *Penicillium* and *Stachybotrys*, may result in pulmonary inflammation (Eduard 2009 and Pucheu-Haston et al 2010).

Different strains of *S. chartarum* have the ability to readily release mycotoxins, potentially posing serious health risks from exposure after long after the actual flooding event (Straus 2009; Charpin-Kardouch et al 2006). Chemotype S produces atranones, and chemotype A produces the more potent and potentially harmful macrocyclic trichothecenes (ILS 2004). Spores from *Stachybotrys chartarum* and *Aspergillus versicolor* have both been associated with increased production of reactive oxygen species and subsequent oxidative DNA damage, even after limited exposures (Rakkestad et al 2010).

Stachybotrys-associated trichothecenes, ranging from <0.2 ppb to 18 ppb, were isolated from human urine, mucus and biopsied tissues after environmental mold exposure; aflatoxin levels, ranging from 1 to 5 ppb, were also detected in the non-control samples (Hooper et al 2009). Trichothecene levels were significantly elevated in more than half of the sera of residents in *Stachybotrys*-contaminated buildings (Brasel et al 2004). This apparently holds true for non-humans as well, after two cats residing in a flood-damaged home had detectable levels of an *S. chartarum* biomarker, Satratoxin G, present in their blood (Mader et al 2007). Satratoxin G-albumin adducts were present in blood serum in

both humans and rats tested after known exposure to *S. chartarum* (Yike et al 2006). These adducts, along with IgE antibodies, may serve as potential biomarkers to indicate exposure to *Stachybotrys* (Van Emon et al 2003; Vojdani 2005). IgA antibody levels in saliva after mold exposure may also act as potential biomarkers, as residents in moldy homes had higher levels of mold and mycotoxins-specific IgA antibodies than controls (Vojdani 2003b). People exposed to molds and/or mycotoxins may exhibit comparable pulmonary and cognitive deficiencies as those exposed to strong chemicals (Kilburn 2009).

Mold remediation and occupational exposures

Occupational exposure to mold spores may occur in remediation workers, farmers, construction workers, sewage workers and other professions requiring the disturbance of organic material. Workplace exposures to mold have been implicated in the development of new-onset adult asthma (Karvala et al 2010; Mäkelä et al 2011). For farmers, handling old plant material and working in enclosed structures, such as greenhouses, may result in exposures to high levels of bioaerosols (Hansen et al 2011; Madsen et al 2009; Skórska et al 2005). Fungal spore counts and endotoxin levels in breathing zones of Polish composting plant workers and waste collectors were higher than 4 mg/m^3 and 10 ng/m^3, respectively (Krajewski et al 2001). Pulmonary complications, defined as emphysematous-like diseases, were evident in employees whose office building possessed high levels of *Stachybotrys chartarum* and concentrations of *Aspergillus versicolor* up to 10,000 spores/m^3 (Hodgson et al 1998). *S. chartarum* contamination in

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an office building was associated with upper and lower respiratory symptoms, along with a reduction in mature T-lymphocyte cells (Johanning et al 1996).

Professional remediation workers are often exposed to high concentrations of mold spores; however, little is known about the exposure of non-professionals remediating mold-infested homes. In Rautiala et al's study, both repair and demolition of moldy homes resulted in high concentrations of airborne microorganisms, ranging from 10^5 to 10^6 CFU/m^3 for viable mold and 10^4 CFU/m^3 for Actinobacteria (1996). Demolition of a fire-damaged building eight days after the event resulted in the release of more than 10,000 spores/m^3 (Rautiala et al 2002). Potentially pathogenic, slow-growing mycobacteria may be released when construction workers remediate both moldy and non-moldy buildings, as concentrations 160 CFU/m^3 were evident (Rautiala et al 2004). Three different methods of reducing mold spores during mold remediation included: local exhaustion common in asbestos removal, negative pressure and room isolation, and a portable exhaust fan with a side draft hood; local exhaustion was the best for mold spore reduction (Rautiala et al 1998).

Syncephalastrum, a typically non-pathogenic mold, was found in a New Orleans resident's bloodstream; the participant performed mold remediation work without PPE on the day of sampling (Rao et al 2007b). Studies regarding mold remediation and the use of N95 respirators indicated that professional remediation workers and residents fail to consistently wear proper respiratory protection; lack of compliance was often due to lack

of training in proper fit, respirators not provided by employers or general discomfort (CDC 2006; Cummings et al 2006; Cummings et al 2007)

In addition to microbial exposures, remediation workers may also be exposed to antimicrobial chemicals. Some cleaners used for residential mold removal are: Lysol All-Purpose cleaner, Fantastik Orange, general household bleach and Orange Glo multipurpose degreaser (Menetrez et al 2007). Professional remediators may also use advanced techniques, such as the application of titanium dioxide, peroxide, chlorine dioxide gas, gamma irradiation, ozone, germicidal bleach, steam cleaning, Borax, Jomax, Killz, Sterifab, Zinase and baking soda media blasting (Adhikari et al 2010; Foster et al 2011; Melandro 2011; Wilson et al 2004; Wilson et al 2005) The cleaners may have moderate to high efficacy in the reduction of microbial growth and removal, sometimes even on porous building materials such as sheetrock (Fukuzaki 2006; Krause et al 2006; Rutala and Weber 1997; Yang 1972). The regular use of sodium hypochlorite, bleach, in cleaning households of some asthmatic children significantly reduced the frequency of asthma attacks (Barnes et al 2008). However, the Environmental Protection Agency still advocates using mild detergent and water to clean non-porous moldy surfaces and discarding moldy porous items (EPA 2011). Due to the caustic nature of the antimicrobial agents, exposure may cause upper and lower respiratory irritation, wheezing, coughing, asthma and dermal burns (USHHS 2011; Jaakkola and Jaakkola 2006; Quirce and Barranco 2010).

IV. Hypothesis and Objectives

The main hypothesis of the study is that the microbial concentrations and compositions of air in New Orleans households were different in pre-Katrina and post-Katrina environments.

The secondary objectives were:

1) To characterize airborne mold exposure pre-Katrina from comparing two mold spore sampling impactors

2) To establish if significant correlations existed between fungal and bacterial markers in dust from previously water-damaged New Orleans homes and characteristics of the homes

3) To determine if total and specific Immunoglobulin E (IgE) antibodies positively correlated with indoor mold exposure duration and severity in New Orleans homeowners and workers.

V. Methods and Materials

Basic Research Design

The main purpose of this research was to determine if microbial concentrations and composition of air and dust in New Orleans households were different in pre-Katrina and post-Katrina environments. Post-Katrina data, hereafter, will be designated as 'early post-Katrina' (fungal and bacterial marker data) and 'late post-Katrina' (biomarker exposure data for residents and remediation workers), although samples were collected in 2007-08 and 2009, respectively.

My role in the pre-storm research consisted of database construction and data analysis. Pre-Katrina, the objectives were: to determine the types and concentrations of cultured and non-cultured fungi in air and potential mold exposures and to assess the airborne mold spore collection efficiency of a newly released viable mold impactor (BioCassette) in relation to an oft-used impactor (Andersen).

Early post-Katrina, fungal (mycotoxins and ergosterol) and bacterial (endotoxins-LPS, endotoxins-LAL and muramic acid) markers in New Orleans house dust were analyzed. In collaboration with the University of Cincinnati, air and additional dust samples collected from the same 15 homes were analyzed for $(1\text{-}> 3)\text{-}\beta\text{-D-glucans}$ and endotoxins (bioactivity-LAL); air samples from 3 of the homes were selected by U.C. for analysis of non-viable fungal spores (Adhikari et al 2010). Health questionnaires were administered to 14 of the homeowners during the HUD study.

Late post-Katrina, potential sources of mold exposure were analyzed in 3 different household media: mold spores in air, the presence of *Aspergillus versicolor* or *Stachybotrys chartarum* in dust and mold antibodies (total and specific IgE) in blood serum. Questionnaires pertaining to home characteristics and reported health effects were used to ascertain potential health effects due to mold exposure.

Study Participants

A majority of the participants in the research study were selected from Orleans Parish (Greater New Orleans area); an exception was made for one Jefferson Parish resident pre-Katrina and two residents post-Katrina (Table 2). Most areas were inundated with at least 0.7 meters (2 feet) of flood water, with districts such as Lower Ninth Ward, Gentilly, Lakeview and New Orleans East receiving more than 2.3 meters (7 feet) (HUD 2005). Pre-Katrina, the four participants were all patients recruited from a local asthma clinic. The 3-home group consisted of a parent and 16-year-old child, an 11-year old child, and an adult. Post-Katrina, dust samples were collected from 15 homes for analysis of microbial concentrations. Fourteen homeowners responded to a health-related questionnaire, as part of a larger, HUD-funded study with the University of Cincinnati. For inclusion in the HUD study, the home must have received at least one-half foot of water or had evidence of significant moisture intrusion due to post-storm flooding and/or wind damage.

Table 2. Study Homes and Flooding by Planning District

Planning District	Total Number of homes in district (% flooded > 0.7m)	Pre-Katrina (n=3)	Early Post-Katrina (n=15)	Later Post-Katrina (n=12)
Algiers	20,053 (0)	0	0	1
Bywater	18,027 (49)	0	3	0
Central Business (CBD)	1,183 (5)	1	0	0
Garden	24,000 (19)	0	1	2
Gentilly	17,343 (82)	0	6	4
Jefferson Parish	Unavailable	1	0	2
Lakeview	11,722 (83)	0	0	1
Lower 9th Ward	7,138 (87)	0	1	0
Mid-City	35,582 (67)	0	0	1
New Orleans East	27,986 (79)	0	4	1
Uptown	29,853 (42)	1	0	0

Source: Planning district information and flood depths (HUD 2005)

For late post-Katrina data, there were 21 participants and 13 houses (including 1 house sampled twice in different stages of repair). The participants were: 6 laborers, 1 volunteer, 5 control homeowners and 9 non-control homeowners, and all were over 18 years old. Volunteers under the age of 18 were permitted to participate as long as written parental consent was given; however, all volunteers who elected to participate were at least 18 years old. There were 4 non-damaged homes (controls), 6 renovated homes and 2 non-renovated homes (1 of which became a partially renovated home during sampling).

Definitions of remediation status were determined by the researchers using modified

versions from a prior study (Table 3) (Adhikari et al 2010).

Table 3. Definitions of remediation status for post-Katrina homes

Remediation Status	Definition
Non-damaged	No moisture intrusion from extensive roof damage or flooding
Renovated	Damaged sheetrock and flooring replaced
Partially Renovated	Gutted; sheetrock potentially replaced, but mold or damage visible
Non-renovated	Ungutted; damaged sheetrock and flooring still in room

A blood sample was not obtained from the homeowner of the second non-

renovated/partially renovated house, due to scheduling conflicts. The homeowner

completed a questionnaire and consented to pre-and post-gutting environmental

sampling. After the blood samples were obtained, there were 2 dropouts, leaving a total

of 19 participants prior to the environmental sampling. One control homeowner and one

of the two non-renovated homeowners, who resided in a separate unit on the same

premises, voluntarily dropped out. The data consisted of questionnaire responses from all

21 participants, total and specific IgE blood serum from 20 participants and

environmental sampling results from 13 homes (the sole non-renovated/partially

renovated home in different stages of repair was counted twice).

Data collection procedures

Pre-Katrina, 47 total indoor and 9 outdoor air samples were collected in 3 homes. Early post-Katrina, there were 31 dust and 15 indoor air samples analyzed from 15 homes. Late post-Katrina, 26 indoor air, 13 outdoor air and 13 dust were collected from 13 homes; blood samples were obtained from 20 participants. Pre- and post-Katrina health data were collected using questionnaires.

Pre-Katrina

A Single Stage N6 Andersen Cascade Impactor (Thermo Fisher Scientific, Franklin, MA) and a BioCassette (EMLab, Phoenix, AZ) loaded with 2% Malt Extract Agar (MEA) were operated side-by-side simultaneously in each living room and master bedroom to collect viable mold samples. An additional bedroom was sampled in the third home, which housed both an asthmatic adult and child. The devices were operated for 1-, 2- and 3-minute intervals to collect 28.3L, 56.6L and 84.9L of air, respectively (no 3-minute living room sample was collected for House 3). Outdoor air samples were collected for 2 minutes approximately 1 meter from each front door; exception: outdoor viable sampling time frame was 3 min. for House 3. An Air-O-Cell cassette (Zefon International, Ocala, FL) was used in each sampling room and outdoors for five consecutive minutes at 15 L/min. to measure fungal spore counts. Air-O-Cell sampling was conducted for 2 min. outside of each home. BioCassette and Andersen samples were shipped to EMLab (California) for culturing and analysis of colonies, and Air-O-Cell cassettes were shipped to EMLab P&K (New Jersey) for analysis of fungal spores.

Early Post-Katrina

For the post-Katrina microbial marker dust assessment, the following were selected: 6 renovated homes, 6 partially renovated homes and 4 non-renovated homes (includes the ungutted upper level of a partially renovated home). The flooring material at the testing sites consisted of wood, concrete slab, carpet, tile, linoleum or a small rug on concrete. Prior to dust collection, the moisture level in the flooring was measured using a GE® Protimeter MMS Plus (General Electric Company Measurement and Control Solutions, CA). In each house, samples were collected from one room with considerable mold growth or water damage (if damage was still present). If neither sign of biological contamination was evident at the time of sampling, the designated sampling site was chosen using either the homeowner's recollection of prior mold or water damage sites pre-renovation or the homeowner's preference for living room or bedroom sampling.

Dust samples were collected from a $1m^2$ area in the center of the floor in each home for 5 minutes following the Housing and Urban Development (HUD) Dust Sample Collection Protocol (Ashley 2006). A Filter Queen Majestic vacuum cleaner (Health-Mor Industries, OH) and small single-use filters were used to collect dust, which was secured in small, zipper-closure plastic bags pre-labeled with each home's study ID. Due to the heavy sediment caked onto the hard floors and the very dusty conditions in four of the homes, four dust samples were collected using a small dustpan and an accompanying plastic-bristled broom instead of a vacuum (Bloom et al 2009a). To avoid cross-contamination of samples, the dustpan and the brush were washed with a mild detergent and water, sprayed with 70% ethanol and allowed to fully dry after using at each home.

In a single home, a sample was collected in the ungutted, upper-level carpeted master bedroom from a $1m^2$ area in the center of the room, and an additional broom-and dustpan bulk sample was obtained from the wood flooring in the gutted living room downstairs for same-house comparison. In the field, collected samples were placed in a specimen cooler containing frozen icepacks before storage at -20°C in the lab. Post collection, all dust samples were shipped to the University of Lund in Sweden for analysis of the potential microbial contaminants.

For the HUD study data, all 15 dust samples were obtained using the same procedures for vacuum collection. However, dust samples were collected at the vacuum's nozzle into conical cloth filter "socks" designed and supplied by the University of Cincinnati. Air samples were collected with an Inhalable Aerosol Button Sampler, containing 25-mm polycarbonate filters and operated with a Leland Legacy personal sampling pump, in the center of the sampling room at a height of 1.1m (SKC Inc, Eighty Four, PA). The button samplers were operated for 4L/min. for approximately 24 hours.

Air and dust samples were placed in ID-labeled plastic bags and put into a cooler filled with ice packs while in the field. In the Tulane laboratory, the filters were removed from the samplers while under the biosafety hood and placed into sterile centrifuge tubes. Air and dust samples were frozen at -20°C and shipped with dry ice to the University of Cincinnati for lab analyses. The samples were analyzed for endotoxin bioactivity (Pyrochrome, Associates of Cape Cod, East Falmouth, MA) and (1->3)-β-D-glucans (Glucatell, Associates of Cape Cod), and 3 of the air samples were also analyzed for non-

viable fungal genera. Follow-up telephone interviews were conducted with 14 homeowners regarding pre- and post-Hurricane Katrina respiratory symptoms, environmental tobacco smoke and exposure to mineral or metal dust.

Late post-Katrina

For post-Katrina mold biomarker exposure data, consent forms and a modified version of two standardized questionnaires, the Centers for Disease Control's post-Hurricane Katrina survey and the European Community Respiratory Health Survey, were administered to all participants by trained technicians prior to blood sample collection (CDC 2006; Janson et al 2001). All homeowners were asked about the following: home re-entry dates for damage inspection or personal item collection, initial cleanup and re-occupancy, use of personal protective equipment at all stages, additional properties gutted, the percentage of cleanup conducted by homeowner versus contractors, genetic sensitivity to mold (atopic allergy) and prior allergy testing and any doctor-diagnosed or undiagnosed respiratory allergies or asthma in the homeowner or other residents. Laborers and volunteers were asked the following: gutting dates and number of homes gutted; visible mold; the use and type of respiratory protection during cleanup; the same respiratory health questions; and consent to allow follow-up contact concerning future health status.

A trained phlebotomist collected all blood samples according to the Head-off Environmental Asthma in Louisiana (HEAL) blood-drawing protocol (personal communication). Blood sample collection was conducted in a non-medical office room

on Tulane University's campus. A 23-gauge Safety-Lok needle with luer adapter was used to draw one blood sample per participant into a 5-mL gold –topped Hemogard^{TM} closure tube containing clot activator and serum separator gel (BD Vacutainer® Venous Blood Collection, Franklin Lakes, NJ). Immediately after each sample was collected, the tube was gently inverted five times and allowed to remain upright at room temperature for 30 minutes to activate the serum separation gel. In the university laboratory, the samples were centrifuged at 1300 x g for 10 min. (Eppendorf® swing-bucket rotor with 4-flex buckets, Hamburg, Germany) to ensure complete separation of the serum from the whole blood and fibrinogen. Three 1-mL aliquots of each serum were drawn, using sterile pipette tips, into 2-mL cryovials. All serum samples were frozen at -20°C and shipped with dry ice to a nationally accredited third-party laboratory for analysis (NIOSH-Allergy and Immunology Division, Morgantown, WV).

In-home visits were conducted 4 to 23 weeks after the blood sample collections. For the laborers who partially renovated one home, air and dust samples were obtained within 4 hours of collecting blood samples pre- and post-gutting. Upon entering each home, a walkthrough and visual inspection was conducted to determine the general environmental conditions. A modified version of the HEAL study's environmental sampling protocol was followed (personal communication-manual). Either the participant's bedroom or the living room was selected as the sampling site, according to the preference of the homeowner or evidence of mold and water damage. The temperature and relative humidity in the room and 1 meter from the home's front door were measured with a thermohygrometer pen (Thermo Fisher Scientific, Franklin, MA). A Strait-line Sonic

Laser Tape 50 (Strait-line, Huntersville, NC) was used to measure the area of the

sampling room. A Surveymaster® protimeter (GE Company, Longmont, CO) was used

to measure the moisture levels of the sampling room's walls and floor. Two Air-O-Cell

cassettes and pumps (Zefon International, Ocala, FL) were placed side-by-side on tripods

approximately in the center of the sampling room at a height of 1m and operated

simultaneously for 10 minutes at 15 L/min. An outdoor Air-O-Cell sample was collected

approximately 1 m outside of the front door.

A Mighty-Mite canister vacuum cleaner (Eureka, Bloomington, IL) with single-use

allergen filters (EMLab P&K, Phoenix, AZ) entrained in the nozzle was used to collect

two dust samples per home over a five-minute period, according to the HUD Dust

Sample Collection Protocol (HUD, 2004). Two additional bulk dust samples, one from

the kitchen and the other from the living room, were collected from the dusty floors of

the partially renovated home, using a small dustpan and broom instead of the vacuum

(Bloom et al 2009). Air samples were placed in ID-labeled plastic bags and stored at

room temperature until shipped to an accredited third-party laboratory (EMLab P&K,

Phoenix, AZ). Dust samples were stored at -20°C in ID-labeled plastic bags before

shipment to an accredited third-party laboratory (INDOOR Biotechnologies,

Charlottesville, VA). Ten residual dust samples collected to meet the microbial marker

exposure objective in 2007/08 were also submitted to the INBIO lab in 2009 for

Aspergillus and *Stachybotrys* ELISA testing.

Laboratory Instrumentation, Techniques and Reagents

Air

Mold spore analysis

In determining the pre-Katrina and post-Katrina fungal exposures, EMLab/ EMLab P&K, respectively, analyzed the Air-O-Cell cassettes for non-viable mold spores. Endotoxins and $(1->3)$-β-D-glucans in the air samples were analyzed by the University of Cincinnati HUD study collaborators. The cassettes contain glass slides coated with a sticky substance on which the mold spores and other particulates adhered, when connected to the Zefon Bio-Pump Plus® (Zefon International, Ocala, FL). In the lab, the slides were viewed with a microscope after staining, and no more than 100 spores of each fungal genus were directly counted in the total traversed sections perpendicular to the slide (Baxter et al 2005). Spore counts were given as spores/m^3, based on the raw fungal counts and the volume of air drawn for the sample. *Penicillium* and *Aspergilllus* spores are not readily distinguishable and are grouped together in lab analyses.

Mold culturing and colony analysis

Mold spores collected with viable samplers, such as BioCassette and Andersen, adhere to the agar in the petri dishes. Incubation at the lab (EMLab) takes five to seven days, during which time the spores are allowed to grow; lab analysts identified and quantified fungal colonies according to fungal species (Jensen and Schafer 1998). Data were reported as colony-forming units per cubic meter (CFUs/m^3).

Dust

Measurement of fungal and bacterial markers

The dust samples were homogenized and divided into portions for the different analyses at the University of Lund in Sweden. Erg, MA, and LPS were determined by GC-MSMS using an ion-trap instrument as described previously (Sebastian et al 2004). In brief, aliquots of the dust samples were subjected to acid (LPS, MA) respectively alkaline (Erg) hydrolysis, purified, and derivatized prior to analysis; LPS was calculated by summarizing the number of moles of the found 3-hydroxy acids and dividing by four (Sebastian et al 2004). Separate dust aliquots were extracted with methanol; after purification, a portion of each extract was analyzed by HPLC-MSMS, using a triple quadrupole instrument, for SATG, SATH, GLIO, AFLAB1, and STRG, whereas a remaining portion of the methanolic extract was derivatized and analyzed by triple quadrupole GC-MSMS for VER and TRID (Bloom et al 2009a). Finally, separate aliquots of the dust samples were extracted with a buffer and subjected to analysis for endotoxin bioactivity using a chromogenic Limulus method (Mårtensson et al 1997).

Chromogenic Limulus amebocyte lysate (LAL) assay was used to measure biological activity of endotoxins in post-Katrina dust by both University of Cincinnati (Associates of Cape Cod) in late 2008 and by the University of Lund in Sweden in early 2009. The HUD study's lab also used (1->3)-β-D-glucan-specific kinetic chromogenic LAL assay (modified version of the endotoxin-LAL that uses Glucatell reagent) (Adhikari et al 2010). For all 3 analyses, either sodium hydroxide or hydrochloric acid was added to the dust sample to ensure that the pH was between 6.0 and 8.0. Fifty microliters of dust was

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mixed with reconstituted LAL reagent from horseshoe crab amebocytes and incubated at 37°C for 10 minutes (Lonza 2008). A substrate solution was added to the mixture and incubated for 6 extra minutes. A stop reagent, made of sodium dodecylsulfate (SDS), was used to halt the reaction. The presence of endotoxin was confirmed in the sample if the solution was yellow, which occurred due to the release of p-nitroaniline (pNA) from enzymatic reactions. A spectrophotometer measured the absorbance, which was directly proportional to the endotoxin concentration, at 405-410 nm. The calibration standard consisted of *Escherichia coli* endotoxin. HUD endotoxin dust data were converted from EU/m^2 used by the University of Cincinnati to EU/mg, for comparison with the Lund-analyzed data (Equation 1) (Dawson 1995).

Equation 1. Conversion of EU/m2 to EU/mg- Endotoxin data

$$EU/mg = \frac{(EU/m^2)}{(1g/m^2)} = EU/g = \frac{(EU/g)}{(1g/1000mg)}$$

Source: Dawson 1995

Enzyme-linked Immunosorbent Assays (ELISA) and MARIA™ (Multiplex Array for Indoor Allergens)

Post-Katrina dust samples were analyzed at INDOOR Biotechnologies for comparison of 2 species-specific environmental molds with antibodies found in blood sera. The dust was first passed through a No. 45 mesh screen to remove large debris and fibers, when necessary (Dr. Bryan Smith, 2009 personal communication). 100 mg of fine dust was then extracted with 2.0mL of Phosphate Buffered Saline (PBS)-5% Tween at room temperature (Earle et al 2007; Smith-p.c.). The extract was centrifuged at 2500 rpm for

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20 minutes to pelletize the dust in preparation for the assay. All samples were then tested at a 1:2 dilution with *Stachybotrys chartarum* and *Aspergillus versicolor* ELISAs.

In ELISA, a binding protein specific to the antigen of interest is coated onto the well bottoms, samples are added and antigenic proteins bind to the substrate (Campbell 2002). After several rinses with buffering solution, a mouse capture protein containing enzyme (usually either peroxidase or alkaline phosphatase) adheres to any bound sample antibodies; the enzymes release a color whose intensity is directly proportional to the amount of antibody present. Dust samples from two homes were tested with Alt a 1 ELISA (*Alternaria alternata* allergen), and MARIA^{TM} was used to test for Der p 1 (one dust mite allergen). For ELISA testing, the dust was sieved through a Number 45 mesh screen to remove larger particulates and fibers; 100 mg of fine dust was extracted with 2.0 mL PTS-T, 0.05% buffer and analyzed starting from a 1:2 dilution (Dr. Bryan Smith, personal communication). The process for MARIA was similar to ELISA, except that it could simultaneously measure up to 8 common allergens using a single Universal Allergen Standard (Earle et al 2007). 5.6 μm polystyrene microspheres internally labeled with fluorescent dyes were covalently coupled with capture monoclonal antibodies, which were then analyzed for fluorescence of the specific allergens of interest via lasers in a Luminex xMAP®. The results were expressed as the median of 100 measurements of each allergen.

Blood serum

ImmunoCAP® (FEIA- Fluoroenzymeimmunoassay): This technique was used by a CDC/NIOSH lab to test the post-Katrina blood sera for both total and specific IgE. The process was similar for both tests, except the specific IgE in this case was for 10 select mold allergens. The process was similar to those used in conventional ELISA testing. Mouse monoclonal antibodies (anti-human IgE), covalently coupled to ImmunoCAP substrate, reacted with the Total or Specific IgE present in the serum sample (ImmunoCAP 2010; Lam 2008). A washing solution, comprised primarily of purified water and a small amount of Phadia's "Component A" to make 'Solution A', was used to remove any unbound antibodies. Enzyme-labeled antibodies against IgE (mouse monoclonal antibodies labeled with β-galactosidase) were then added to complex with the bound IgE. These complexes were incubated for 120 minutes and rinsed again with washing solution to remove unbound enzyme-anti-IgE. The complexes were developed with a solution made of 4-methylumbelliferyl-β-D-galactosidase for 60 minutes before they were washed with solution and the reaction was stopped with 4% sodium carbonate. The microarrays were then read with a Phadia UniCAP 100® instrument; the fluorescence of the eluate was directly proportional to the amount of IgE in the serum sample. Calibration curves were generated using human IgE from an unbroken line linked to the 2nd International Reference Preparation (IRP) 75/502 Human Serum Immunoglobulin E from the World Health Organization (WHO).

Statistical analyses

SPSS was used for all statistical analyses (IBM SPSS Statistics, vers.19.0, Somers, NY), unless otherwise noted. Microsoft Excel was used to calculate geometric means for the glucan and endotoxin data (Microsoft Excel, Office 2007, Redmond, WA). All data were determined to be non-normally distributed based on Quantile-Quantile plots. Statistical significance was determined at the 10% level for all hypotheses. However, p-values below 5% were determined to be strongly statistically significant, whereas p-values between 5% and 10% were classified as weakly statistically significant.

Descriptive statistics

Arithmetic mean, median and interquartile ranges were calculated for valid observations of fungal and bacterial markers, for total IgE and post-Katrina Indoor/Outdoor mold spore counts and for common variable data. Taxa-specific and overall spore and colony frequencies and ranges were determined pre- and post-Katrina. Boxplots were generated for late post-Katrina Total IgE levels.

Chi square exact tests

This statistical method was used to determine proportional similarities among the categorical variables pre- and post-Katrina (Equation 2). Exact significance p-values were used, instead of asymptotic, due to the small sample sizes.

Equation 2. Chi Square (χ^2) test

$$\chi^2 = \Sigma \frac{(\text{Observed frequency} - \text{Expected frequency})^2}{\text{Expected frequency}}$$

Source: Key 1997

Pre-Katrina, questionnaire data (pertaining to mold growth, pet ownership, air conditioning, environmental tobacco smoke, hospitalization and respiratory health) were compared, based on mean indoor/outdoor (I/O) culturable fungal colony counts and mean I/O non-culturable fungal spore counts. For post-Katrina environmental fungal and bacterial markers, the test was used to compare fungal markers (mycotoxins and ergosterol), bacterial markers (endotoxin-LAL, endotoxin-LPS, carbon chain lengths of 10 to 18 atoms and muramic acid) and home characteristics (water-damaged vs. non-water-damaged homes, visible mold and/or water damage during sampling, floor material and floor moisture). The Chi square test was also performed on the same variables according to dust collection method, vacuum vs. broom and dustpan. Mold exposure, respiratory health, mask usage and I/O spore counts were compared based on total IgE.

Ratios

Indoor/outdoor (I/O) airborne mold spore ratios were calculated pre- and post-Katrina (Equation 3). Ratios of "1" or greater indicate that indoor spore counts equal or exceed outdoor levels. Ratios were calculated for Erg, LPS and MA early post-Katrina, according to home remediation status.

Equation 3: Mold ratio (spores/m^3 or CFU/m3) = $\dfrac{[\text{Indoor mold count}]}{[\text{Outdoor mold count}]}$

Odds ratios, based on the probability of our study population to report respiratory distress or have elevated total IgE after potential exposure to indoor mold, were calculated for pre- and post-Katrina research and for late post-Katrina data, respectively.

Equation 4: Odds ratio $= \dfrac{[P(\text{factor} \mid \text{exposed}) / (1 - P(\text{factor} \mid \text{exposed})]}{[P(\text{factor} \mid \text{unexposed}) / (1 - P(\text{factor} \mid \text{unexposed})]}$

Source: Kendziorski 2011

Non-parametric tests:

Spearman correlation

This statistical method, equivalent to the independent t test, was also used to determine associations between the aforementioned variables. Exact significance was calculated. This test was also used to determine correlations between our endotoxin-LAL data and the HUD-analyzed endotoxin-LAL data. Data were interpretated as the following: low correlation = 0.3 – 0.5; moderate correlation = 0.5 – 0.7; and high correlation = 0.7 to 0.9.

Equation 5: Spearman rho $(\rho) = \dfrac{(1 - 6\Sigma d^2)}{[n(n^2-1)]}$

n = number of paired ranks; d = difference between paired ranks

Source: Calkins 2005

Mann Whitney U test

Mann Whitney test, a non-parametric equivalent to independent samples t test, was used to determine group differences pre- and post-Katrina. Pre-Katrina, the mold collection efficiencies of the BioCassette and Andersen were compared. Mann Whitney was also used to determine differences between microbial marker levels (based on remediation status) early post-Katrina.

Equation 6: $U = \{N_1N_2 + [N_1(N_1+1)]/2\} - R_1$

$U =$ Mann Whitney statistic; N_1 and $N_2 =$ number of samples in each of 2 groups; $R_1 =$ sum of the first group's ranks

Sources : IFA *n.d.*; Leech et al 2008

Wilcoxon Signed Rank test

This test, an alternative to the related samples t test, was used to determine differences between the laborers' pre-and post-gutting sera total IgE levels.

Equation 7: $Z = \dfrac{K - \text{Mean}(K)}{\text{SD}(K)} = \dfrac{K - (n/2)}{\sqrt{(n/4)}}$

$K =$ positive differences between the two groups; n= number of non-zero difference data pairs

Source: Iowa State University 2002

Kruskal Wallis test

This test, equivalent to the One-way Analysis of Variance, was used for early post-Katrina data to determine if the microbial markers differed according to remediation status.

Equation 8: $K = \{N-1\}\dfrac{[k\Sigma j=1\ (C_j D_j) - N[(N+1)/2]^2}{[m\Sigma i=1\ (A_i R_j) - N[(N+1)/2]^2}$

C_j=number of observations in column j; D_j=average rank of column j observations; A_i=number of row i observations; R_i=average rank of row I observations

Source: U.S. Geological Survey 2002; Leech et al 2008

Timeline

Pre-Katrina air samples were collected in July 2004. Early post-Katrina dust samples and HUD study air and dust were collected from November 2007 to February 2008. HUD study air and dust samples were analyzed by the University of Cincinnati in August 2008, and early post-Katrina dust samples were analyzed by the University of Lund (Sweden) in January 2009. Post-Katrina blood samples were drawn in April and May 2009, and residential environmental air and dust samples were obtained from June 2009 until October 2009. Lab analyses occurred from November 2009 until March 2010. The manuscript for the post-Katrina microbial marker data was submitted to *Indoor Air* journal on September 20, 2011. A modified version of the manuscript, included in this dissertation, was re-submitted to *Science of the Total Environment* journal on December 16, 2011. The manuscript for the pre-Katrina data was submitted to *Environmental Research* journal on October 14, 2011, and the manuscript for the later post-Katrina

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blood and environmental data was submitted to *Science of the Total Environment* journal also on October 14, 2011.

Research Limitations

The primary limitation for the overall research was the very small sample size. The number of homes sampled ranged from 3 to 16, and the number of participants ranged from 4 to 21. Due to the limited number of participants, collecting more samples over a longer timeframe might have increased the strength of the studies. However, the pre-Katrina research was abruptly halted by the unexpected arrival of Hurricane Katrina a year later. Post-Hurricane Katrina research might have yielded more statistically significant data if the samples were collected within months of the storm, as opposed to 2 to 4 years later. Personal bioaerosol sampling, i.e. attaching button samplers to the lapels of the residents and workers might have been more applicable to individual mold exposures, especially for the pre- and post-gutting exposures of the laborers.

Recall bias may be an issue, as questionnaires were administered 3 to 4 years after the storm. It is difficult to determine exactly when and where mold exposures occurred, and one post-Katrina participant unexpectedly overlapped in the "renovated homeowner" and "laborer" categories. Sample collection methods should have been more uniform pre- and post-Katrina, i.e. using a viable mold spore sampler post-storm, recruiting more asthmatic patients post-Katrina and contacting pre-Katrina participants for post-Katrina sampling. Post-Katrina household dust could have been analyzed with mold-specific quantitative polymerase chain reaction (MSQPCR) to compare our fungal data with the Environmental Relative Moldiness Index (ERMI).

VI. Results for Pre- and Post-Katrina Questionnaire Data

Questionnaire data for pre-and post-Katrina are summarized in Table 4. A child was

defined as anyone less than 18 years of age, and lung trouble includes pulmonary issues

other than asthma (i.e., Chronic Obstructive Pulmonary Disorder or bronchitis). Doctor-

diagnosed conditions (dd) are indicated in parentheses.

Table 4. Summary of pre- and post-Katrina questionnaire data

Category	Pre-Katrina (BioCassette vs. Andersen) (n=4)	Early Post-Katrina (Microbial markers/ HUD) (n=14)	Late Post-Katrina (Mold biomarkers) (n=21)
Characteristics			
Environmental tobacco smoke	3	2 (10-18 cigs/day)	N/A
Floor moisture (At-risk/Wet)	N/A	8	3
Mold and/or water damage	2	8	2
Pets	1 (bird)	1 (dog)	5 (8 dogs; 1 cat)
Demographics			
Adults/Children	2 adults; 2 children	14 adults	21 adults
Female/Male	3F; 1M	10F; 4M	14F; 7M*
Respiratory Health			
Allergies (dd)	0	0	7 (3-Yes)
Asthma (dd)	4	0	1 (Yes)
Coughing	4	2	3
Difficulty breathing	4	2	3
Elevated Total IgE	N/A	N/A	8 (1-mold-specific)
Lung trouble	4-pre; N/A-post	0-pre; 1-post	0
Medication use	3	0	1
Wheezing	4	2	3

*This was not a question on the late post-Katrina survey. Classification according to gender was based solely on perceived physical attributes for this group.

Respiratory Symptoms Pre-and Post-Hurricane Katrina

Before Hurricane Katrina, three of the four participants (2 children under the age of 18 and 1 adult, all with doctor-diagnosed asthma) responded that they required at least daily use of asthma medications, such as a nebulizer or inhaler (Table 4). The non-familial adult and child were both hospitalized twice in the 12 months prior to the survey for breathing difficulties.

Twelve of the 38 participants pre- and post-Katrina reported symptoms of respiratory distress, including coughing, wheezing, and difficulty breathing (Table 5). For this research, those who were exposed to buildings with visible mold and/or water damage were only half as likely to report symptoms of respiratory distress than unexposed persons (Equation 9).

Table 5. Contingency table for odds of respiratory distress & mold exposure

Factor (mold exposure)	Respiratory distress symptoms		
	RD +	RD -	Total
Damage +	5	15	20
Damage -	7	11	18
Total	12	26	38

*Damage = visible mold and/or water damage in personal residence or home (s) gutted

Equation 9. Odds ratio for post-Katrina respiratory distress & mold exposure

$$OR = \frac{5/15}{7/11} = \frac{0.3}{0.6} = 0.5$$

After Hurricane Katrina, 7 of the 35 post-storm participants reported having either

doctor-confirmed or unconfirmed respiratory allergies, conditions which were present

before the storm. Three of the participants, 2 renovated homeowners and 1 control

homeowner, noted having exacerbated respiratory symptoms (primarily sneezing and

wheezing) after Hurricane Katrina, ranging from sometimes to frequently. All three

participated in post-storm cleanup and gutting, and two reported staying in a FEMA

trailer for at least a year or two after the storm. None of the participants had been

hospitalized in the prior 12 months for breathing problems.

Pre-Katrina questionnaire data were compared with mean I/O colony and spore counts.

Categories included: mold growth (leaks and/or visible mold), pet ownership, air

conditioning (window or central), environmental tobacco smoke, respiratory health

(frequency of medication use), hospitalization in the prior 12 months from asthma

symptoms and age (child under 18 years of age or adult) (Cummings et al 2008). Exact

chi square tests and Spearman correlations calculated for all categories, based on mean

I/O colonies and mean I/O spore counts, determined that there were no statistically

significant differences (0.05 < p > 0.10) (Table 6).

Table 6. Exact chi square (χ^2) tests and Spearman (ρ) correlations based on
fungal counts

Category	Chi square statistic (p-value)		Spearman rho (p-value)	
	Viable I/O	Non-viable I/O	Viable I/O	Non-viable I/O
Age	3.615 (1.0)	1.752 (1.0)	-0.447 (0.67)	0.000 (1.0)
Hospitalization	3.615 (1.0)	1.987 (1.0)	0.894 (0.33)	-0.707 (0.67)
Mold Growth	3.667 (1.0)	1.752 (1.0)	-0.258 (1.0)	0.000 (1.0)
Pet ownership	3.667 (1.0)	1.752 (1.0)	0.258 (1.0)	0.000 (1.0)
Respiratory Health	7.112 (1.0)	5.936 (0.17)	0.632 (0.50)	-1.000 (0.17)
Smokers in home	3.667 (1.0)	3.138 (0.50)	-0.775 (0.50)	0.816 (0.50)
Ventilation	3.615 (1.0)	1.987 (1.0)	0.894 (0.33)	-0.707 (0.67)

Pre- and post-Katrina home characteristics are shown in Table 7. Post-Katrina homes were grouped according to remediation status, defined as: 1) Non-damaged= no moisture intrusion; 2) Renovated= damaged sheetrock and flooring replaced; 3) Partially renovated= gutted (sheetrock potentially replaced, but mold or damage still evident); and Non-renovated= ungutted. Partially renovated and Non-renovated 'n' include 2 homes in different stages of repair twice early and late post-Katrina.

Table 7. Overall home characteristics

Classification	Characteristic	Pre-Katrina (n=3)	Post-Katrina Remediation Status*			
			Non-damaged (n=4)	Renovated (n=12)	Partially Renovated (n= 7)	Non-renovated (n= 6)
Building frame	Brick	1	0	4	2	2
	Wood	2	4	8	5	4
Building style	Single-story	1	3	11	4	3
	Split-level	2	1	2	3	3
Building type	Apartment/Condo	3	0	0	0	0
	Single-family home	0	4	12	7	6
Chemical Treatment	Untreated	--	--	2	4	4
	Treated	--	--	10	3	2
Damage	Visible mold	2	--	--	3	6
	Water Damage only	1	--	--	4	0
Flood Status	Flooded (range in m)	--	--	1.3 – 3	0.2 – 3.3	1 – 3.3
	Roof Damage only	--	--	0	1	1
Floor Moisture Status	Dry	--	4	6	4	4
	At-Risk	--	0	6	3	1
	Wet	--	0	0	0	1
Floor Type	Carpet	--	1	1	0	3
	Tile	--	1	6	0	0
	Wood	--	2	4	4	2
	Other	--	0	1	3	1
Residence Status	Not occupied	0	0	4	4	5
	Occupied	3	4	8	3	1
Thermohygrometry	Relative Humidity (%)	40 - 62	38-58	39-74	43-78	37-74
	Temperature (°C)	22 - 28	24-28	16-30	16-28	17-27

VII. Airborne Fungal Taxa Results

Pre-Katrina

Primarily, non-viable fungal taxa detected in indoor and outdoor air samples pre-Katrina

were: basidiospore and ascospore groups and *Penicillium/Aspergillus* and *Curvularia*

genera (Figure 4). Indoor detection frequencies ranged from 100% for basidiospore

groups to 0% for Alternaria, Chaetomium and Stachybotrys. The remaining spores were

from *Bipolaris/Drechslera* group, *Cladosporium*, *Ganoderma*, *Nigrospora*, *Pithomyces*,

Smuts/Periconia/Myxomycetes, *Stemphylium* and *Zygosporium* genera.

Figure 4. Pre-Katrina non-viable fungal taxa frequencies
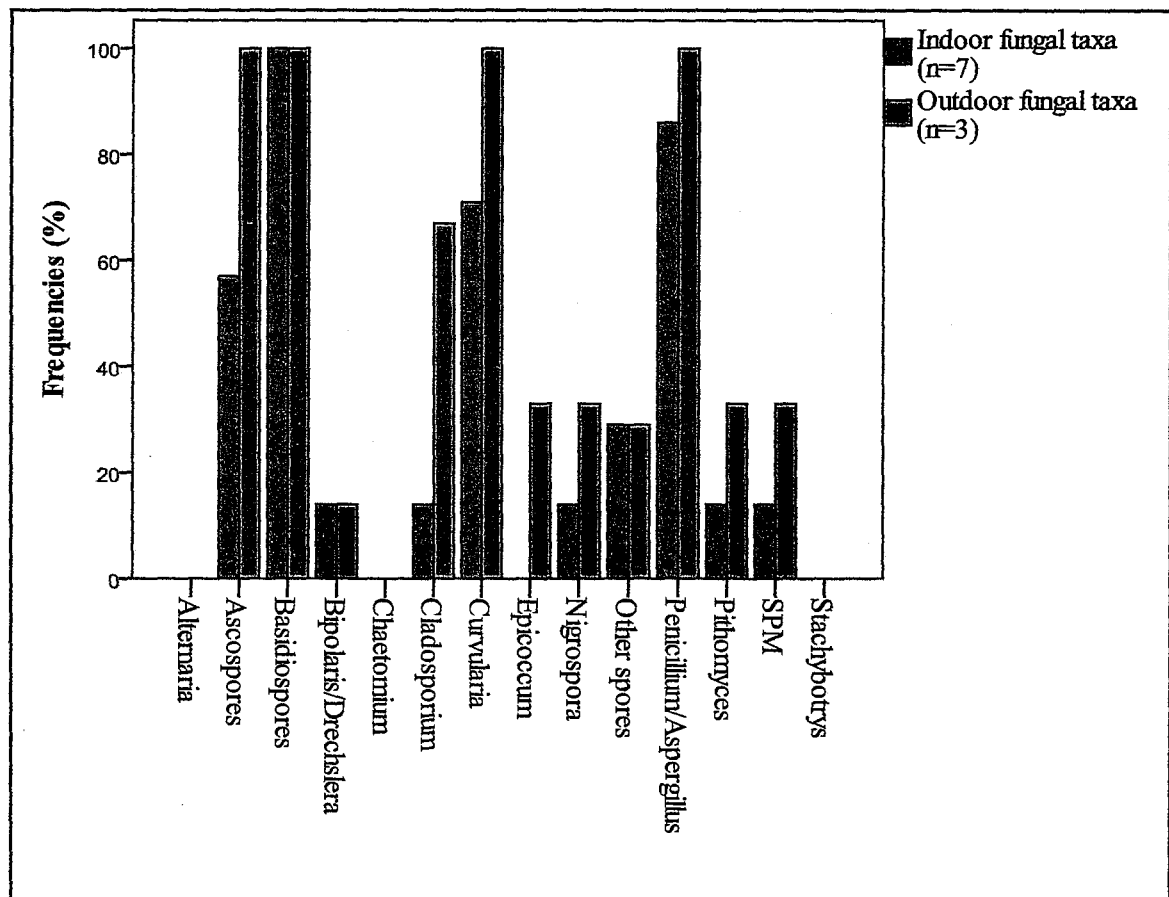
Indoor viable mold colonies predominantly consisted of *Aspergillus* species,

Cladosporium, *Mucor*, Non-sporulating colonies and *Penicillium* (Figure 5). Indoor

detection frequencies ranged from 3% to 8% for most taxa. The remaining colonies

consisted of *Bipolaris/Drechslera*, *Fusarium* and *Mucor*. Other mold genera and species

detected both indoors and outdoors included: *Aureobasidium pullulans*, *Epicoccum*,

Paecilomyces, *Syncephalastrum* and *Verticillium*. Two mold species, *Aspergillus*

fumigatus and *Aspergillus versicolor*, were only found in indoor air samples.

Figure 5. Pre-Katrina viable fungal taxa frequencies
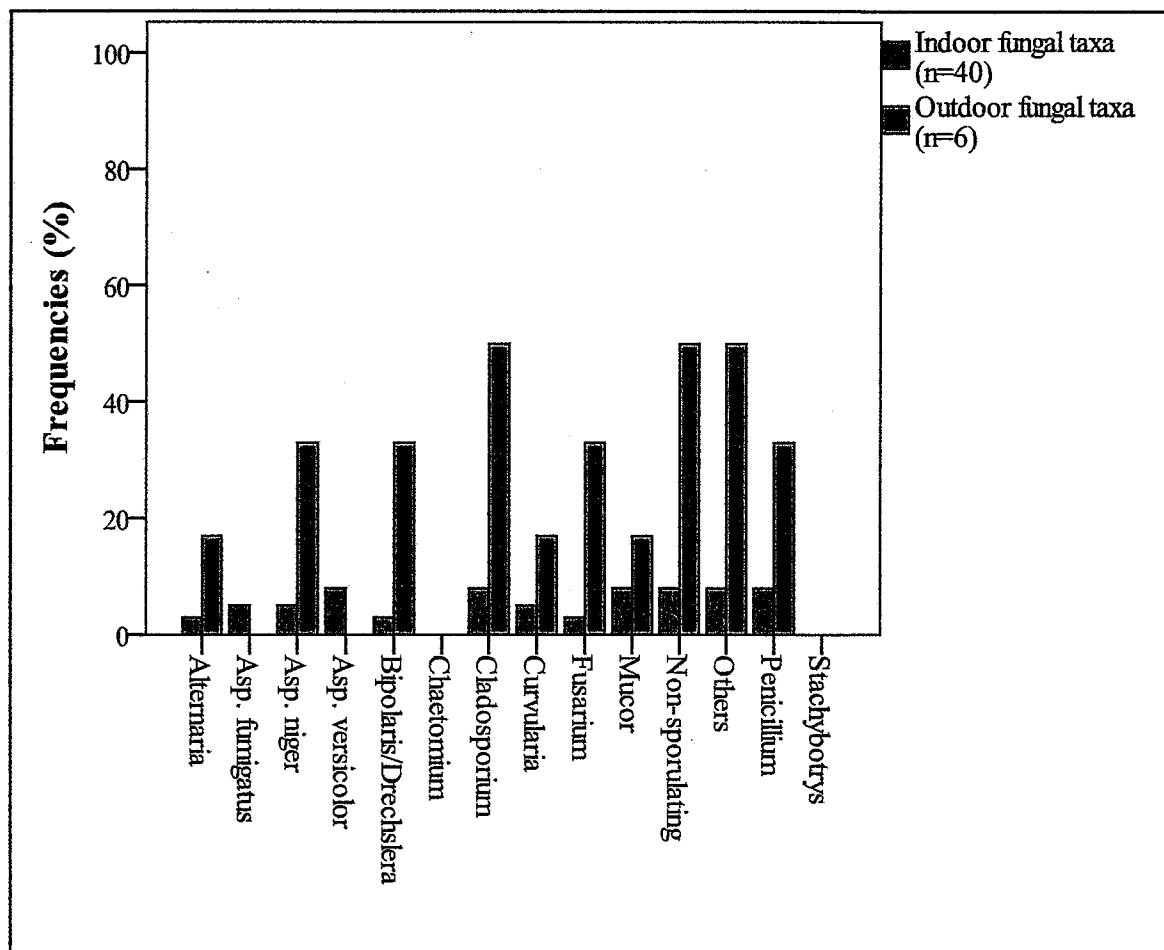
Mean indoor/outdoor (I/O) fungal taxa ratios were generated for all 3 sampling devices

(Table 8). Parenthetical numbers represent percentages of total indoor colonies or counts

by commonly detected fungi. Mean ratios for BioCassette and Andersen impactors

include only 2-minute sampling frames for 2 homes and a 3-minute sampling frame for

the third home (due to different outdoor sampling lengths). Indoor Air-O-Cell data

(collected for 5 minutes per room) were divided by 2.5 as an approximation of spores

collected in 2 minutes; this permits I/O counts to be generated for comparison.

BioCassette and Andersen data are in colony-forming units per cubic meter (CFU/m^3),

and non-viable Air-O-Cell data are in $spores/m^3$. All *Pencillium* data also includes

Aspergillus; spores from the two genera are difficult to distinguish microscopically, so

both categories were combined for viable data as well for consistency.

Table 8. Mean Indoor/Outdoor Mold Ratios and Indoor Percentages

Fungal Taxa	BioCassette (n=9)	Andersen (n=9)	Air-O-Cell (n=10)
Overall	1.09	0.88	0.08
Ascospores	n/a	n/a	0.03 (6)
Basidiospores	n/a	n/a	0.06 (60)
Cladosporium	1.45 (17)	1.24 (15)	0.02 (1)
Curvularia	--	6.42 (5)	0.42 (6)
Non-sporulating	1.06 (18)	0.48 (23)	n/a
Penicillium/Aspergillus	5.29 (53)	2.66 (38)	0.10 (22)

*n/a = not applicable for this method;
**'--' = unable to calculate (not detected outdoors)

Spearman correlations were computed for non-viable spores and their species-specific

viable colony counterparts (Table 9). Non-sporulating colonies were compared to non-

viable spores in the 'Other' category. None of the p-values was statistically significant

$(0.05 < p > 0.10)$.

Table 9. Spearman exact correlations for viable and non-viable fungal taxa

Fungal Taxa	Spearman rho (ρ) (p-values)
Cladosporium	0.180 (0.62)
Curvularia	0.371 (0.29)
Penicillium/Aspergillus	-0.396 (0.26)
Non-sporulating/Others	0.482 (0.16)

Mann Whitney testing was conducted for BioCassette and Andersen mold spore

collection efficiencies. There was no statistically significant difference between the two

devices (U = 137.0; p = 0.22); therefore, it was concluded that the BioCassette may

recover culturable mold as efficiently as the Andersen.

Post-Katrina

Non-viable fungal taxa detected in indoor and outdoor air samples post-Katrina

predominantly were: basidiospore and ascospore groups and *Penicillium/Aspergillus* and

Cladosporium genera (Figure 6). Indoor detection frequencies of these fungi ranged from

33% to 100% early post-Katrina to 50% to 54% in late post-Katrina samples. Spores in

the 'other' category were from *Cercospora, Ganoderma, Torula* and unidenitifed genera.

Stachybotrys spores were found in 1 non-renovated home and in 2 outdoor air samples

late post-Katrina. *Chaetomium* spores were detected in 6 indoor air samples (2 from a

renovated home and 4 from the same home in non-renovated and partially renovated

stages).

Figure 6. Post-Katrina non-viable fungal taxa frequencies
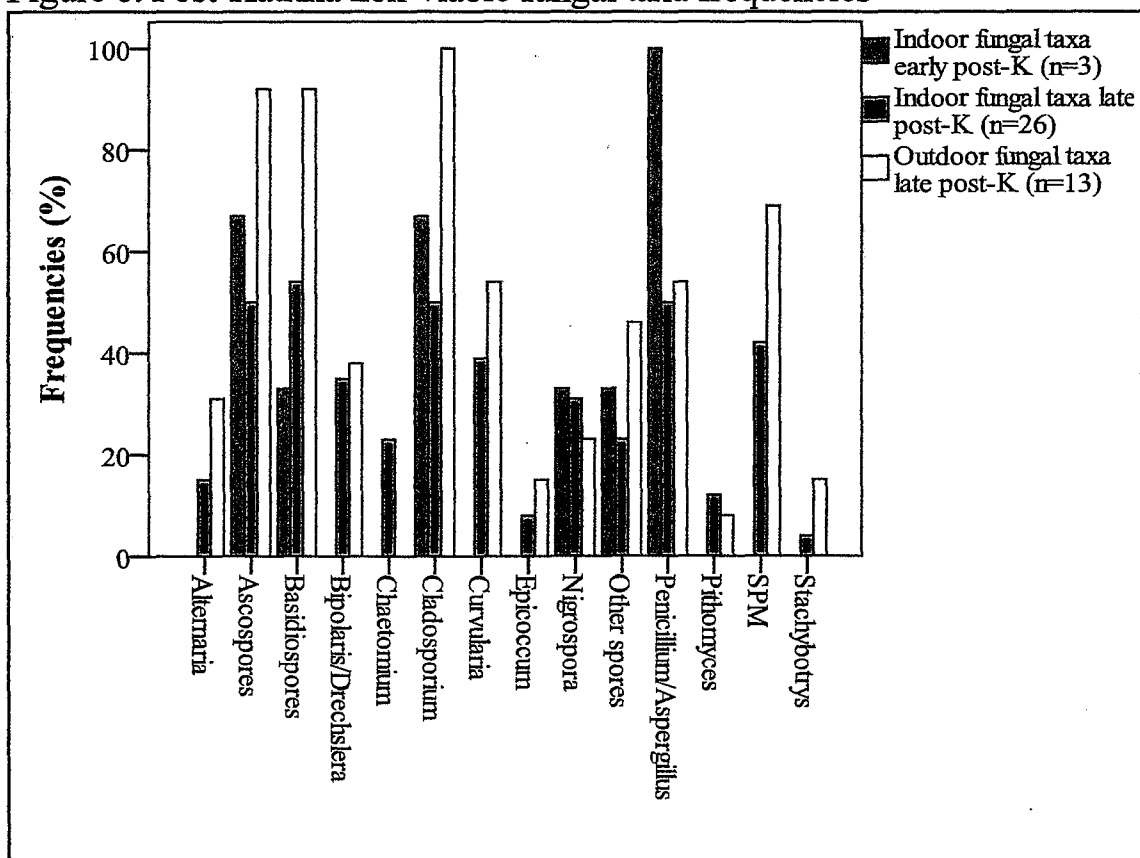
Table 10 shows the proportions of leaf surface fungi in pre-and post-Katrina indoor air samples. Non-viable air samples did not have detectable levels of leaf surface fungi, excluding taxa comprised of undifferentiated leaf surface and soil fungal spores. For the pre-Katrina viable air samples, 55% had between 20 to 80% LSF, with most concentrated toward the mid- to lower end of the range. Early post-Katrina air sample data collected yielded 67% of samples with <20% LSF (10 and 17%), and only one sample within the 20-80% range (44% LSF). Half of the late post-Katrina air samples collected with Air-O-Cell cassettes exhibited at least 20% LSF, with 53% of the LSF found in renovated home samples. For post-Katrina control homes, 75% of the samples did not have any detectable levels of LSF.

Table 10. Proportions of leaf surface fungi in pre- and post-Katrina indoor air

Group	Number of samples (n)	Number of cases for each proportion of leaf surface fungi in indoor air			
		0%	< 20%	20-80%	> 80%
Pre-Katrina Non-viable	7	2	5	0	0
Pre-Katrina Viable	40	9	9	22	0
Post-Katrina Non-viable (early)	3	0	2	1	0
Post-Katrina Non-viable (late)	26	9	4	9	4
Non-damaged Homes	8	6	0	0	2
Renovated Homes	12	2	3	6	1
Non-renovated Homes	4	1	1	1	1
Partially renovated Home	2	0	0	2	0

Total airborne fungal taxa counts pre- and post-Katrina are summarized in Table 11.

There are no established federal regulations; however, general guidelines were mentioned

in the literature review. For overall non-viable indoor counts before the storm, total spore

counts (5,280 spores/m^3) exceeded Baxter et al's general guideline of a 'clean' home (<

1,200 spores/m^3). Outdoor non-viable mold spore levels were 'high' (27,700 spores/m^3)

pre-Katrina based on the National Allergy Bureau data, primarily due to basidiospore

counts. Pre-Katrina indoor viable mold colony total counts (12,676 CFU/m^3) were more

than 10 times greater than the higher-end of the recommended ACGIH guidelines (1,000

CFU/m^3), primarily due to *Penicillium* species. Post-Katrina non-viable spore counts

("FAMB") overall greatly exceeded the indoor guidelines (117,596 spores/m^3), although

the count was largely skewed due to non-renovated (101,758 spores/m^3) and renovated

(11,688 spores/m^3) air samples; non-damaged homes (150 spores/m^3) were more

representative of 'clean' homes. Post-Katrina outdoor non-viable counts were also 'high'

(42,870 spores/m^3), due to elevated *Aspergillus/Penicillium* levels.

Table 11. Pre- and post-Katrina total fungal counts

Fungal Taxa	Pre-Katrina non-viable samples (spores/m³)		Pre-Katrina viable samples (CFUs/m³)		Post-Katrina non-viable samples (spores/m³)		
	Indoors (n=7)	Outdoors (n=3)	Indoors (n=40)	Outdoors (n=6)	Indoors (n=3)	Indoors (n=26)	Outdoors (n=13)
Alternaria	0	0	0-82	0-53	0	0-47	0-47
Ascospores	0-160	1,300-1,900	N/A	N/A	0-237	0-347	0-1,300
Aspergillus/Penicillium	0-370	930-2,300	N/A	N/A	131-1,484	0-32,000	0-25,000
A. fumigatus	N/A	N/A	0-18	0	N/A	N/A	N/A
A. niger	N/A	N/A	0-177	0-82	N/A	N/A	N/A
A. versicolor	N/A	N/A	0-65	0	N/A	N/A	N/A
Penicillium	N/A	N/A	0-5,313	0-753	N/A	N/A	N/A
Basidiospores	210-850	4,400-10,000	N/A	N/A	0-5	0-2,200	0-3,900
Bipolaris/Drechslera	0-13	0-33	0-267	0-124	0	0-157	0-27
Chaetomium	0	0	0	0	0	0-1,500	0
Cladosporium	0-53	0-930	0-1,869	71-647	0-421	0-60,900	27-2,600
Curvularia	0-93	33-170	0-254	0-24	0	0-267	0-100
Epicoccum	0	0-33	0	0	0	0-13	0-7
Fusarium	0	0	0-330	0-128	0	0	0
Mucor	N/A	N/A	0-479	0-12	N/A	N/A	N/A
Nigrospora	0-13	0-100	0	0	0-165	0-270	0-47
Non-sporulating	N/A	N/A	0-1,378	124-753	N/A	N/A	N/A
Others	0-53	0-300	0-153	18-36	0-118	0-2,030	0-73
Pithomyces	0-13	0-33	0	0	0	0-27	0-47
Smuts/Periconia/Myxomycetes	0-13	0-130	N/A	N/A	0	0-630	0-300
Stachybotrys	0	0	0	0	0	0-13	0-60
Total Count	**5,280**	**27,700**	**12,676**	**3,046**	**2,868**	**117,596**	**42,870**
Overall I/O	**0.19**		**4.16**		**N/A**	**2.74**	

VIII. Microbial markers and Biomarkers

Early post-Katrina

Descriptive statistics for fungal and bacterial marker dust data are summarized in Table 12. Of the 7 mycotoxins analyzed, satratoxins G and H and gliotoxin were not detected in any samples; only a trace amount of aflatoxin B1 was found in a single sample. Gliotoxin was detected in only a quarter of the samples. Sterigmatocystin, predominantly produced by *Aspergillus versicolor*, was present in half of the samples; levels ranged from trace amounts to more than a thousand pg/g dust.

Statistical analyses for microbial markers are presented in Table 13. Kruskal Wallis tests, based on remediation status, were significant for 3-OH fatty acids C:12 to C:18 and for muramic acid ($p < 0.05$). Mann Whitney U tests showed significant differences between renovated and non-renovated homes for lipopolysaccharides (LPS), C:12 – C:18 (four components of LPS), and muramic acid (MA). Renovated and partially renovated homes showed significant differences between LPS, C:10 to C:16 and MA. Partially renovated and non-renovated homes showed significant differences only for C:16 and C:18. Non-renovated home mean ranks were greater than partially renovated and renovated, and partially renovated home mean ranks exceeded renovated mean ranks for these parameters.

Table 12. House dust fungal and bacterial markers- descriptive statistics

Classification	Type	n	Mean	Median	Interquartile Range
Fungal	Ergosterol (ng/mg) (N=16)	16	9.4	10.0	5.3 – 11.5
	Mycotoxins (pg/g) (N=16)				
	Aflatoxin B1(AFLAB1)	1	0.2	0.2	--
	Gliotoxin (GLIO)	0	--	--	--
	Satratoxins G and H (SAT G/H)	0	--	--	--
	Sterigmatocystin (STRG)	8	1359.5	4.6	2.3 – 830.7
	Trichodermol (TRID)	4	3.4	3.4	0.9 – 5.9
	Verrucarol (VER)	5	435.2	137.6	3.4 – 272.3
Bacterial Gram Negative	Endotoxin-LAL (EU/mg) (N=16)	16	90.0	80.0	42.5 – 135
	Endotoxin-LPS (pmols/mg) (N=16)	16	44.0	39.8	22.0 – 54.6
	C:10	16	7.8	2.9	1.8 – 11.2
	C:12	16	22.8	16.9	8.9 – 35.6
	C:14	16	47.7	38.7	16.9 – 57.0
	C:16	16	55.2	55.4	28.1 – 75.3
	C:18	16	42.4	31.1	19.6 – 66.5
Gram Positive	Muramic acid (ng/mg)(N=16)[a]	13	19.7	14.7	6.9 – 34.2

*Abbreviations: Limulus amebocyte lysate (endotoxin-bioactivity); LPS- lipopolysaccharide
**'--'= not applicable
[a] Muramic acid - analysis failed in 3 samples

Table 13. Kruskal Wallis and Mann Whitney tests for microbial markers

Classification	Type	n	Kruskal Wallis (χ^2)	Mann Whitney (U)
Fungal	Ergosterol (ng/mg) (N=16)	16	χ^2= 3.930 (0.14)	U= 5.000 (0.17)
	Mycotoxins (pg/g) (N=16)		--	--
	Aflatoxin B1(AFLAB1)	1	--	--
	Gliotoxin (GLIO)	0	--	--
	Satratoxins G and H (SAT G/H)	0	--	--
	Sterigmatocystin (STRG)	8	χ^2= 0.718 (0.72)	U= 10.500 (0.86)
	Trichodermol (TRID)	4	$\chi 2$=1.650 (0.50)	U= 8.000 (0.47)
	Verrucárol (VER)	5	χ^2= 0.022 (0.99)	U= 12.000 (1.0)
Bacterial				
Gram Negative	Endotoxin-LAL (EU/mg) (N=16)	16	χ^2= 4.151 (0.12)	U= 3.000 (0.06)
	Endotoxin-LPS (pmols/mg) (N=16)	16	χ^2= **9.647 (0.002)**	U= 0.000 (0.01)
	C:10	16	χ^2= 3.107 (0.22)	U=10.000 (0.76)
	C:12	16	χ^2= **8.443 (0.007)**	U= 0.000 (0.01)
	C:14	16	χ^2= **9.283 (0.003)**	U= 0.000 (0.01)
	C:16	16	χ^2= **10.426 (0.001)**	U= 0.000 (0.01)
	C:18	16	χ^2= **9.107 (0.004)**	U= 0.000 (0.01)
Gram Positive	Muramic acid (ng/mg)(N=16)[a]	13	χ^2= **6.945 (0.02)**	U= 0.000 (0.04)

* Kruskal Wallis and Mann Whitney tests grouped by remediation status = χ^2 or U (p-value); significance: **p < 0.05**; LAL (p < 0.10)

**Abbreviations: Limulus amebocyte lysate (endotoxin-bioactivity); LPS- lipopolysaccharide

***'--'= not applicable

[a] Muramic acid - analysis failed in 3 samples

Microbial marker median ratios and 95% confidence intervals are presented in Table 14. Statistically significant differences, inter- and intra-group based on remediation status, were observed for Erg/LPS, MA/LAL and MA/LPS. For Erg/LAL, significant differences were seen overall and for renovated and non-renovated groups. For Erg/MA, only differences within the non-renovated group were statistically significant.

Table 14. Microbial marker median ratios by remediation status

Markers	Overall	Renovated (n=6)	Partially renovated (n=6)	Non-renovated (n=4)
Erg/LAL	**0.2 (0.1 – 0.2)**	**0.2 (0.1 – 0.2)**	0.1 (0.0 – 1.0)	**0.2 (0.1 – 0.2)**
Erg/LPS	**0.2 (0.1 – 0.4)**	**0.3 (0.1 – 0.7)**	**0.2 (0.1 – 0.6)**	**0.2 (0.1 – 0.2)**
Erg/MA	0.4 (0.3 – 1.2)	0.8 (0.2 – 1.3)	0.3 (0.3 – 1.5)	**0.4 (0.3 – 0.4)**
LPS/LAL	0.6 (0.3 – 1.0)	0.6 (0.2 – 1.2)	0.4 (0.3 – 1.8)	0.9 (0.7 – 1.4)
MA/LAL	**0.3 (0.1 – 0.5)**	**0.2 (0.1 – 0.5)**	**0.3 (0.1 – 0.9)**	**0.4 (0.2 – 0.5)**
MA/LPS	**0.4 (0.3 – 0.6)**	**0.4 (0.4 – 0.6)**	**0.5 (0.3 – 0.8)**	**0.3 (0.3 – 0.5)**

Chi square exact tests for home characteristics were calculated based on remediation status (Table 15). Remediation status was weakly statistically significant for flooring moisture ($p = 0.05$); renovated homes had more 'at-risk' moisture readings than either partially renovated or non-renovated homes. However, stronger significance was observed for remediation status and flooring type ($p = 0.01$) and remediation status and visible mold and/or water damage ($p = 0.002$). Wood flooring was observed in at least one home in each category for remediation status. It was expected that the visible mold and/or water damage variable would differ between the groups; as defined in this research, a renovated home would not have visible mold or water damage. However, all non-renovated homes had both mold and water damage present at the time of sampling.

Spearman exact correlations between home characteristics and microbial markers were assessed (Table 16). Low correlation coefficients ranged from 0.3 to 0.5, moderate from 0.5 to 0.7, and high from 0.7 to 0.9. Weak statistically significant data had p-values greater than 0.05, but less than 0.10; strong significance indicated a p-value less than 0.05. Moderately strong correlations were observed between visble mold and/or water damage and the following markers: LPS, C:12 to C:18 and the use of a chemical treatment (non-renovated and partially renovated homes were primarily associated with lack of treatment). Highly strong correlations were observed between visible damage and muramic acid, remediation status and C:18 and with remediation status and muramic acid. Renovated homes were associated with lower levels of muramic acid and C:18. There were moderately weak positive correlations between visible damage and ergosterol and negative correlations between flooring moisture and muramic acid ('dry' floors in non-renovated homes were associated with higher levels of muramic acid). Low weak correlations were associated between gram-negative bacteria bioactivity and the following: occupancy status, building frames and remediation status. Occupied homes, wooden frame houses and non-renovated homes were associated with higher levels. Low weak negative correlations were observed between flooring type and gram-negative bioactivity (homes with carpet and concrete had lower endotoxin bioactivity), and occupancy status and sterigmatocystin (occupied homes had lower sterigmatocystin levels). A moderately strong negative correlation was observed between temperature and verrucarol; lower temperatures were associated with higher levels of verrucarol.

Table 15. Chi square (χ^2) exact tests for home characteristics by remediation status

Characteristic	Chi square statistic	p-value
Building Frame	3.233	0.30
Building Style	1.409	0.81
Flood Status	7.685	0.17
Flooring Moisture	8.151	**0.05**
Flooring Type	15.259	**0.01**
Mold and/or water damage	13.388	**0.002**
Occupancy status	2.559	0.33
Treatment status	4.392	0.16

* Significance determined as $(0.10 > p < 0.05)$ (bold)

Table 16. Spearman (ρ) exact correlations for microbial markers and home characteristics[1]

Category	Damage	Flood Status	Floor Moisture	Floor Type	House Frame	House Style	Occupancy	RH	RS	Temp.	Treatment
Fungal											
Ergosterol (n=16)	0.449	--	--	--	--	--	--	--	0.140	--	--
Sterigmatocystin (n=8)	--	--	--	--	--	--	-0.484	--	--	--	--
Trichodermol (n=4)	--	--	--	--	--	--	--	--	--	--	--
Verrucarol (n=5)	--	--	--	--	--	--	--	--	--	-0.535	--
GNB											
Endotoxin-LPS (n=16)	**0.623**	--	--	--	--	--	--	--	0.802	--	--
C:10	--	--	--	--	--	--	--	--	--	--	--
C:12	**0.609**	--	--	--	--	--	--	--	**0.732**	--	--
C:14	**0.652**	--	--	--	--	--	--	--	**0.773**	--	--
C:16	**0.681**	--	**-0.459**	--	--	--	--	--	**0.833**	--	--
C:18	**0.580**	--	--	--	--	--	--	--	**0.755**	--	--
Endotoxin-LAL (n=16)	--	--	--	-0.490	0.435	--	0.440	--	0.427	--	--
GPB											
Muramic Acid (n=13)	**0.738**	--	-0.523	--	--	--	--	--	**0.756**	--	--

[1]. Correlation coefficients are shown in table. Significance: 0.10 > **p < 0.05** (bold)

*Abbreviations: RH-relative humidity; RS- remediation status; Style-single level or split; Temp-temperature (°C)

HUD- and Sweden-analyzed endotoxin data

Endotoxin and (1->3)-β-D-glucan air and dust data from the HUD study were compared

with endotoxin dust data analyzed by University of Lund in Sweden (Table 17). Air

samples were collected for 24 hours at 4 L/min. with button samplers. On average, the

endotoxins present in the dust did not aerosolize as readily as the (1->3)-β-D-glucan

(Adhikari et al 2010). HUD endotoxin dust total count was more than 40 times larger

than Sweden-analyzed dust samples, the latter of which were tested approximately 5

months later. Spearman correlations for the HUD-analyzed endotoxin-LAL dust samples

were not statistically significant for any of the home characteristics; the only statistically

significant association was a moderately strong negative correlation with trichodermol

(rho = -0.591; p = 0.04).

Table 17. (1->3)-β-D-glucans and endotoxin bioactivity early post-Katrina

Descriptives	(1→3)-β-D-glucan		Endotoxin-bioactivity (LAL)		
	Dust (x 10^3 $\mu g/m^2$)	Air (ng/m^3)	Dust (EU/mg)- HUD	Dust (EU/mg)- Sweden	Air (EU/m^3)
N (>LOD)*	10	6	12	14	6
Total	2.43	28.07	50,258	1230	217.16
Median	0.05	0	683	60	0
Mean	0.16	1.87	3,351	82	14.48
Geometric Mean	0.12	4.01	630	59.23	10.89
Interquartile Range	0 – 0.28	0 – 4.54	13.5 – 14.72	30 - 135	0 – 5.51

Early and late post-Katrina dust samples

Ten dust samples collected in the early post-Katrina sampling were analyzed by the

Indoor BioTechnologies lab used for the late post-Katrina analyses. The dust was tested

for the presence of *Aspergillus versicolor* and *Stachybotrys chartarum*, neither of which

was detected in any post-Katrina dust samples. However, two mycotoxins associated with

Aspergillus and Stachybotrys species (sterigmatocystin and verrucarol) respectively,

were detected by the University of Lund in Sweden during the microbial marker analyses

(Table 18).

Table 18. Combined early and late post-Katrina dust samples

	Number of observations		Descriptives		
Group	N	Variable	Mean	Median	Interquartile Range
Post-Katrina (n= 10) pg/g dust	6	STRG (*Aspergillus*)	1811.37	4.90	1.85 – 4365.25
	4	VER (*Stachybotrys*)	475.95	70.50	2.05 – 1355.30

Late post-Katrina

Total Immunoglobulin Type E (IgE) levels in the blood sera of homeowners, laborers and volunteers are categorized in boxplots (Figure 7). Total IgE levels exceeding 100 kU/L are classified as elevated. The black lines in the center of each boxplot represent median values. The 'stem' width for non-damaged/control homeowners is 100.00, and the other groups have stem widths of 10.00.

Figure 7. Boxplots for Total IgE levels in kU/L
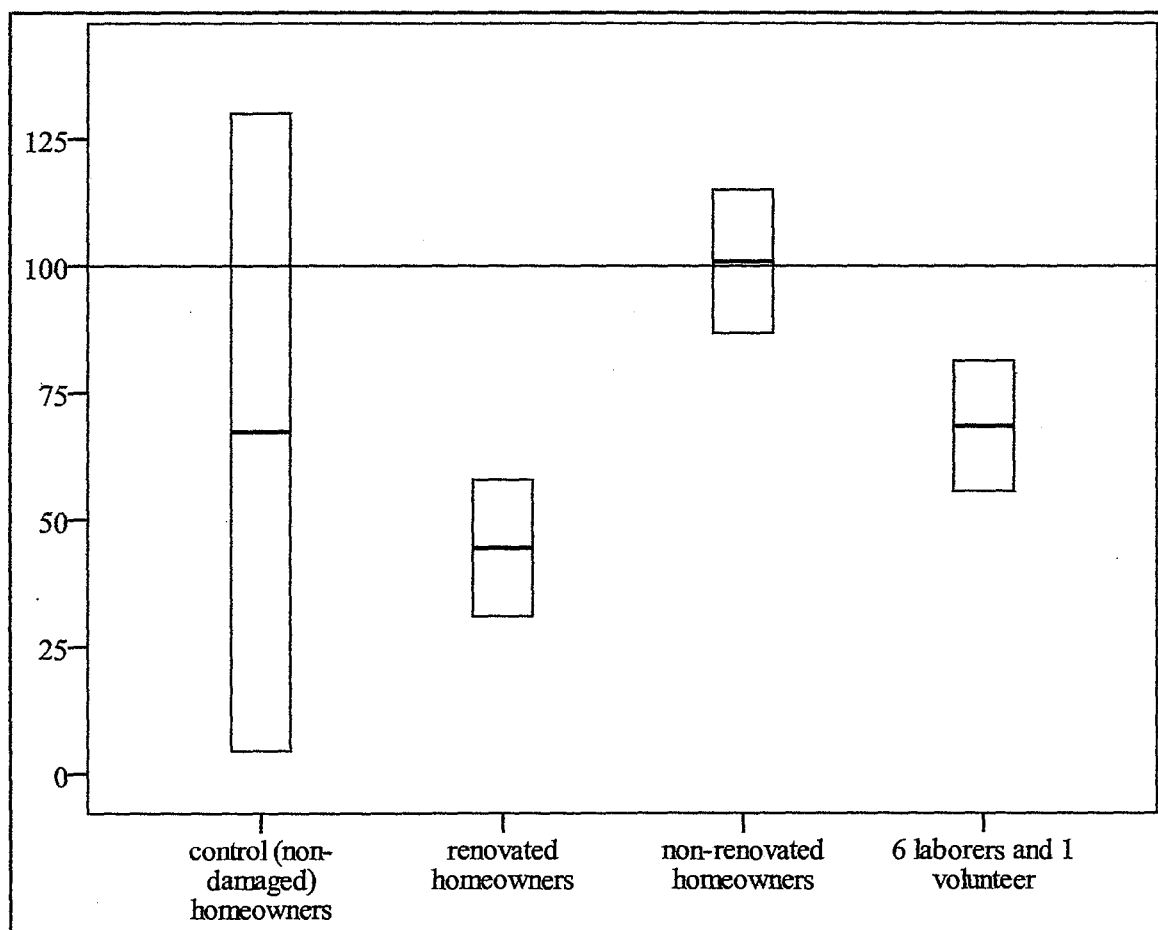
Total IgE levels for all 20 participants were categorized according to 'normal' and 'elevated' levels; mold-specific IgE levels were categorized into classes based on kilounits of antigen per Liter (Table 19). ImmunoCAP organizes Specific IgE results into the following group: Class 0 (<0.35); Class I (0.35-0.70)-Low; Class II (0.7-3.5)-Medium; Class III (3.5-17.5)-High; Class IV (17.5-50)-Very High; Class V (50-99)-Very High; Class VI (≥ 100)-Very High.

The non-renovated homeowners consisted of 2 residents in the same home, one with an elevated IgE level (dropout) and one with a normal level. Three of the five control homeowners had elevated IgE, including the single dropout from the control group. One laborer had elevated IgE levels pre- and post-home gutting, and the sole volunteer also had an IgE antibody level more than the twice the reference point. One renovated homeowner had the highest total IgE level, more than 20 times greater than the reference. The homeowner also had the only positive mold-specific IgE response, with negative responses only to *Aspergillus niger* and *Chaetomium globosum* (Table 20). A high response (Class III) was observed for Alternaria alternata exposure, and medium responses (Class II) were generated for the other 7 mold species. Descriptive statistics for Total IgE and mean I/O ratios are presented in Table 21. Control homeowners had the lowest mean I/O (0.11) and the highest median Total IgE (130 kU/L).

Table 19. Categorized blood sera total and mold-specific IgE antibody levels

| Antibody Test | Measurement | Controls (n=5) | Non-control homeowners (n=8) | | Laborers (n=6) | | | |
			Renovated (n=6)	Non-renovated (n=2)	Pre-gut Laborers (n=5)	Post-gut Laborers (n=5)	Laborer (n=1)	Volunteer (n=1)
Total IgE[b]	Normal (≤ 100)	2	4	1	4	4	1	0
Reference Levels (kU/L)	Elevated (>100)	3	2	1	1	1	0	1
Specific IgE	Class 0 (< 0.35)	5	5	2	5	5	1	1
Evaluation Class (kU_A/L)	Class I -VI (0.35 –100)	0	1	0	0	0	0	0

Table 20. Positive blood serum mold-specific IgE.

sIgE	A. alternata	A. fumigatus	A. niger	C. globosum	C. herbarium	C. lunata	E. purpurascens	F. proliferatum	P. notatum	T. viride
kU$_A$/L	13.7	0.94	<0.35	<0.35	0.87	0.87	2.64	1.74	0.87	0.97
Eval. Class	III	II	0	0	II	II	II	II	II	II

Fungal species, in the following order, are: *Alternaria alternata*, *Aspergillus fumigatus* and *niger*, *Chaetomium globosum*, *Cladosporium herbarium*, *Curvularia lunata*, *Epicoccum purpurascens*, *Fusarium proliferatum*, *Penicillium notatum* and *Trichoderma viride*.

Table 21. Descriptive statistics for control and non-control homeowners, laborers and volunteer

Status	Number of subjects	Descriptives		
Group	n	Variable	Mean	Median	Interquartile Range
Control Homeowner	5	Total IgE (kU/L)	102.91	130.00	10.79 – 181.50
	4	I/O air	0.11	0.07	0.02 – 0.24
Renovated Homeowner	6	Total IgE (kU/L)	449.37	53.35	30.38 – 728.00
	6	I/O air	2.03	1.80	0.92 – 2.78
Non-renovated Homeowner/Partially renovated homeowner	2	Total IgE (kU/L)	100.85	100.85	86.70 – 100.85
	2	I/O air	74.05	9.09	1.06 – 9.09
Laborers and Volunteers	2		147.75	147.75	31.50 – 147.75
Laborers (pre-gut)	5	Total IgE (kU/L)	83.38	55.50	30.5 – 150.15
Laborers (post-gut)	5		84.80	55.60	30.5 - 153.75

Mean Indoor/Outdoor airborne mold spore ratios were calculated based on remediation

status (Table 22). The indoor spore counts consisted of the total number of spores found

in 2 non-viable Air-O-Cell cassette samples per home. Samples less than the detection

limit were counted as one spore per cubic meter. Renovated homes 2 and 6, both non-

renovated homes and the partially renovated home's samples had high background levels

of non-biological debris, and these were regarded as minimum counts. Indoor mold spore

counts were very high in the two non-renovated homes, primarily due to

Pencillium/Aspergillus spores in the first home and *Cladosporium* in the second home.

Table 22. Indoor and outdoor mold spore counts by remediation status

Home Status	Mold Spore Counts (spores/m^3)		
	Indoor (n=26)	Outdoor (n=13)	Mean I/O
Control Homes (n=4)			
CH1	28	410	0.07
CH2	60	210	0.29
CH3	8	1500	0.00
CH4	54	980	0.06
Renovated Homes(n=6)			
RH1	517	310	1.67
RH2	5600	1100	5.09
RH3	54	160	0.34
RH4	1660	1500	1.11
RH5	2216	1100	2.01
RH6	1641	850	1.93
Non-renovated Homes (n=2)			
NRH1	36036	34000	1.06
NRH2 (pre-gut)	65722	310	212.01
Partially renovated Home (n=1)			
PRH (post-gut)	4000	440	9.09

Table 23 shows those potentially exposed to indoor mold due to post-Katrina cleanup of residences and elevated total IgE in blood sera. In this research, those who performed home-gutting were approximately a third as likely to have elevated total IgE as those who were not involved in any home remediation activities (Equation 10).

Table 23. Contingency table for odds of post-Katrina cleanup and total IgE

Factor (mold exposure)	Elevated total IgE in blood		
	tIgE +	tIgE -	Total
Cleanup +	4	9	13
Cleanup -	4	3	7
Total	8	12	20

*Cleanup = gutting of personal residence and/or other moldy homes

Equation 10. Odds ratio for post-Katrina cleanup and elevated total IgE

$$OR = \frac{4/9}{4/3} = \frac{0.4}{1.3} = 0.3$$

A modified variable coding system was used to compare mold exposure (duration and quantity of homes gutted), respiratory health, N95 mask usage, total IgE and I/O spore counts (Cummings et al) (Table 24). Mold exposures were categorized as follows: 0 = no water intrusion in home and no cleanup activities; 1 = no water intrusion in home, but participated in cleanup activities; 2 = water intrusion in home, but did not participate in cleanup activities; 3 = water intrusion in home and participated in cleanup activities; and 4 = participated in cleanup of more than 1 home (laborers and volunteers only). Mask usage during cleanup/gutting was indicated by: 0 = not applicable; 1 = Yes; and 2 = No. Respiratory health responses were assigned scores of: 0 = no respiratory problems reported; 1 = undiagnosed respiratory allergy; and 2 = doctor-diagnosed respiratory allergy. Indoor/outdoor air mold spore ratios were included as: 1 = less than 1 (indoor count below outdoor count) and 2 = 1 or greater (indoor count is equal to or exceeds the outdoor count). Exact Chi square and Spearman rho correlations were used to compare all 4 categories with total IgE levels, which were categorized according to: 0 = not applicable; 1 = normal; and 2 = elevated.

Table 24. Exact chi square (χ^2) tests and Spearman (ρ) correlations for health and exposure data based on Total IgE

Category	Chi square statistic	p-value	Spearman rho	p-value
I/O mold spores	2.793	0.22	-0.502	0.16
Mask Usage	4.645	0.43	-0.133	0.62
Mold Growth	5.761	0.50	-0.161	0.48
Respiratory Health	4.834	0.44	0.043	0.91

No statistically significant differences were for found using either statistical analysis. Sixty-five percent of all participants wore an N95 mask upon initial home inspection post-storm, and 91% of all respondents who participated in gutting activities on their home or other properties used an N95 mask, which apparently did have a protective effect on respiratory health.

Pre- and post-gutting Total IgE data for the 5 laborers were categorized as either 1 = normal or 2 = elevated; Wilcoxon signed rank test was used to determine differences between the pre-and post-gutting serum total IgE levels. There were no statistically significant differences between pre-andpost-gutting IgE (Z = 0.000; p = 1.0).

IX. Discussion

Basidiospores, a fungal group including mushrooms, were found in 100% of pre- and 33% of post-Hurricane Katrina homes sampled. Spores from the *Basidiomycota* phylum have been present for decades throughout New Orleans' environment and may contribute to respiratory concerns (Salvaggio et al 1973). To date, no other pre-storm environmental data for New Orleans households are available. Our research study focused on assessing microbial markers and potential pre- and post-Katrina mold exposures from a multimedia perspective, including questionnaires, blood serum, air and dust samples.

For viable sampling, EMLab found no statistically significant difference in the collection efficiencies of the BioCassette and Andersen cascade impactor (Purves and Georgianna 2003). We also concluded that the BioCassette may have been as efficient at recovering culturable mold as the Andersen. BC consistently exhibited the most CFUs/m^3. All 3 homes had small areas of visible mold and/or water damage. Although there are no mold standards, the overall levels of culturable mold in the homes fell outside of the recommended ACGIH indoor fungal guidelines of 100 – 1000 CFU/m^3 (Jo et al 2005). This may have been attributable to inadequate air exchange in the homes during the relatively hot and humid temperatures in late July 2004 or from active mold growth indoors.

Pre-Katrina, the predominant fungi cultured were: *Penicillium*, Non-sporulating colonies, *Cladosporium* and *Curvularia*. The first three fungal taxa were consistent with predominant culturable mold found in air in United States' homes, regardless of

geographic location (Shelton et al 2002). Non-viable spores primarily were: basidiospores, *Penicillium/Aspergillus*, ascospores, *Curvularia* and *Cladosporium*. Background debris, i.e. non-biological matter and other structures, may have hindered the analyst's ability to properly view all spores on the slides (Jensen and Schafer 1998). As noted in another study, it is not unusual for basidiospores and ascospores to be detected in the air, yet remain unlikely to form colonies on MEA (Lee et al 2006). Statistically significant Spearman correlations were not observed for non-viable spores and their species-specific viable colony counterparts, unlike the results found in a similar study (DeKoster et al 1995). However, Zefon's internal study advised against comparing non-viable Air-O-Cell data with those collected by viable samplers (Tsai et al 1998).

Post-Hurricane Katrina New Orleans studies examined viable and non-viable fungal by-products in air and dust, bacterial and dust mite indicators and mold allergy via skin prick testing (Adhikari et al 2009; Adhikari et al 2010; Chew et al 2006; Lee et al 2006; Rabito et al 2010; Rao et al 2007a; Riggs et al 2008; Solomon et al 2006). After Hurricane Katrina, it was assumed that the lack of guidance in practicing proper mold remediation procedures and the failure to wear PPE during all phases of remediation would increase mold exposure (Cummings et al 2006). More than half of our participants wore an N95 mask upon first entering homes post-Katrina and during cleanup. This was consistent with 63% of residents surveyed in a larger post-Katrina study who wore N95 masks while gutting homes (Cummings et al 2008).

The primary fungal genera found in water-damaged New Orleans homes in other studies were soil fungi, particularly *Penicillium/Aspergillus* species, *Paecilomyces*, *Trichoderma* and *Stachybotrys* (Chew et al 2006; Lee et al 2006; Rao et al 2007; Riggs et al 2008; Solomon et al 2006). Trace amounts of water-indicator molds, *Stachybotrys* and *Chaetomium*, were found in and/or around a late post-Katrina renovated home and pre- and post-gutting of NRH2/PR. Both homes had at least 4 feet of flood water post-Hurricane Katrina.

A baseline assessment of New Orleans' microbial environment found a ratio of 4.11 for indoors to outdoors mold spore counts for flood-damaged homes (Schwab et al 2007). Two similar studies found mean indoor/outdoor spore ratios of 5.3 and 8.3, respectively (Solomon et al 2006; Rao et al 2007a). In our research, with the exception of RH3, all of the renovated, partially and non-renovated homes still had indoor airborne mold spore levels that exceeded outdoor spore counts. The median I/O ratio in the non-renovated homes and the partially renovated home was 9.09, which was consistent with levels found in previous studies. Control homes exhibited much lower indoor mold spore counts than outdoors, which was consistent with other homes in southeastern United States without moisture intrusion or visible mold growth (Horner et al 2004; Lee et al 2006).

Pre-Katrina, the total indoor non-viable spore counts for 2 of the homes were consistent with "moldy" homes (both overall spore counts exceeded 2000 spores/m^3) (Baxter et al 2005; NRDC 2005). Total counts for ascospores/basidiospores for the 2 homes also exceeded the suggested guidelines, largely due to the latter fungal group. However, none

of the Penicillium/Aspergillus spore counts in the 3 homes were consistent with "moldy" homes, with levels below 600 spores/m^3. Only one viable indoor air sample, collected from the child's bedroom in House 3 for 1 minute with the BioCassette, exceeded the guidelines (1,269 CFUs/m^3). Overall, outdoor viable colony counts (3,046 CFU/m^3) were less than a quarter of the indoor levels (primarily due to *Penicillium* and non-sporulating colonies).

Post-Katrina non-viable spores collected for microbial marker analysis were within the guidelines for two of the homes (233 and 986 spores/m^3). The third home's total fell outside of the guidelines at 1,649 spores/m^3, which largely consisted of *Penicillium/Aspergillus*. Individually, total spore counts in 8 late post-Katrina non-viable samples exceeded the guidelines, which consisted of samples from a renovated home (RH2), both non-renovated homes (NRH1 and NRH2) and the partially renovated home (previously NRH2). NRH1 spore counts for ascospores/basidiospores (2,213 spores/m^3) and *Penicillium/Aspergillus* (32,000 spores/m^3) were both indicative of a moldy home. The two remaining homes that exceeded the suggested guidelines, based solely on *Penicillium/Aspergillus* counts were RH2 (1,720 spores/m^3) and RH5 (2,130 spores/m^3).

Airborne endotoxins were found in water-damaged New Orleans homes sampled shortly after Hurricane Katrina (Chew et al 2006; Riggs et al 2008; Rao et al 2007a; Schwab et al 2007; Solomon et al 2006). Endotoxins may synergize with mycotoxins and pose additional health risks to some exposed persons (Hirvonen et al 2005; Thrasher et al 2009). Rao et al detected indoor airborne endotoxin and (1->3, 1->6)-beta-D-glucan

levels of 22.3 EU/m^3 and 1.7 ug/m^3, respective geometric means, in water-damaged New

Orleans' homes (2007a). Our HUD study data for airborne endotoxins and (1->3)-beta-D-

glucans were 10.9 EU/m^3 and 4.01 ng/m^3 (0.004 ug/m^3), respective geometric means for

water-damaged homes. Four of the 15 dust samples (27%) actually had an increase in

endotoxin units detected between the initial analysis and the subsequent testing; however,

most endotoxin counts greatly decreased over the 5-month lapse. The slight increase may

have been due to the storage of the Sweden-analyzed samples (maintained at room

temperature for approximately 6 months before storing at -20°C) and the expected

decrease would have been due to the natural degradation of microorganisms over time.

The dust sample with the highest endotoxin count (22, 556 EU/mg) had a mid-range level

during the Sweden analysis (130 EU/mg). Endotoxin bioactivity obviously decreased,

given that the median in dust analyzed by Sweden was down to 60 EU/mg, only 5 months

after the HUD study endotoxin analysis (median of 683 EU/mg).

Moderate to highly significant correlations between microbial chemical biomarkers and

home characteristics were anticipated, due to the oft-cited association between mold,

bacteria and home dampness (Adhikari et al 2010; Kostamo et al 2005; Park et al 2004).

LPS levels, components of gram-negative bacteria membranes, were determined by using

GC-MSMS to analyze dust for 3-hydroxy fatty acids (3-OHFAs), which in our case

included carbon chains from shorter C:10 to longer C:18 chains. Three-hydroxy fatty

acids with C:10 to C:14 lengths may reflect actual endotoxin levels, whereas longer chain

3-OHFAs (C:16 – C:18) may indicate the presence of some Actinobacteria (Sebastian et

al 2005). A slightly higher correlation was observed between the shorter chains (C:12 and

C:14) and the LAL (analyzed by Sweden) than for the longer chains and the LAL, as with

another study (Park et al 2004; Szponar et al 2000). Houses, in this study, with carpet and

wood had higher levels of C:16 and C:18, regardless of flood status, indicating that

Actinobacteria may have been present in at least 44% of the floors sampled.

Endotoxin mean concentrations in one post-Katrina study did not correlate with flood

designation or environment, based on locale (Solomon et al 2006). For our study, the

strongest correlations observed were between visible mold and/or water damage,

muramic acid and LPS (C:12 to C:18, especially C:16), which were primarily seen in

non-renovated homes. For statistically significant marker ratios regardless of remediation

status, homes with higher MA levels also had increased levels of lipopolysaccharides,

indicating that gram-negative bacteria were present in all of the homes, although they

may not have been biologically active when sampled. Endotoxin bioactivity (LAL)

weakly correlated with floor type, occupancy status, remediation status and floor type,

since some renovated and partially-renovated homes actually had higher levels than non-

renovated homes. This may have been attributable to higher levels of remediation activity

in these homes, which may have aerosolized the bacteria and resulted in a lower levels in

dust compared to non-renovated homes; the air in the homes were not tested for

culturable bacteria.

Ergosterol weakly correlated with visible mold and water-damage in partially renovated

and non-renovated homes; some renovated homes also had detectable levels of

ergosterol. It has been noted that increased levels of MA and possibly some endotoxins

may have protective effects for some asthmatic children when exposed early in life (Park et al 2001; Sordillo et al 2010; Sordillo et al 2011; Zhao et al 2008). Erg/MA ratios were statistically significant only for non-renovated homes, which tended to have larger visible patches of mold and water damage than partially renovated homes. LPS, Erg and MA naturally occur in all house dusts, not exclusively in water-damaged, unrepaired homes (Sebastian et al 2003; Sebastian et al 2005).

Mycotoxin production, which is unnecessary for fungal growth, often occurs when fungi are in less than ideal environments; the lack of available nutrients and colder temperatures may be potential factors (Gallup 2006). The heavy weight and volatility of mycotoxins also makes them more prevalent in dust than in air, unlike microbial volatile organic compounds (Korpi et al 2009). *Stachybotrys* is the predominant producer of VER, TRID and macrocyclic trichothecenes, such as SATG and SATH (Bennett et al 2003; Bloom et al 2009a). We found only small amounts of AFLAB1 in a single home, and no SATG or SATH. Although not all fungi will produce mycotoxins in a given environment, any mycotoxins that are produced may potentially elicit an allergic and/or toxic reaction in exposed humans and animals; mycotoxins may also persist in contaminated materials even after the fungi cease to be viable (Bloom et al 2009a; Bloom et al 2009b; Huttunen et al 2004; Metts 2008; Straus 2009). To date, there are no reliable lab tests available to check sera for mycotoxin exposure, other than a potential test for aflatoxins (EHIB, 2009).

A study regarding mycotoxins in post-Hurricane Katrina settled dust in New Orleans homes was conducted by Bloom et al, which detected the presence of VER (600 to 18000 pg/g) and STRG (16000 to 28000 pg/g) in the samples (2009a). However, mycotoxin levels were inconsistent with fungal levels in the study. Another study found mycotoxins and ergosterol in more than half of the dust samples collected from water-damaged buildings in Sweden (Bloom et al 2009b). We detected the most STRG (7552 pg/g dust) in a non-renovated house with the highest level of MA (37.3 ng/mg dust), the second highest Bioactivity-LAL (170 EU/mL) and a 'dry' wooden floor. The second highest level of STRG (3303 pg/g dust) was exhibited in a partially renovated house with the most Erg (22.6 ng/mg dust) and an 'at-risk' slab floor. Both houses flooded post-storm and still had visible mold and signs of water damage, i.e. mud marks on the walls and/or door denoting the height that the floodwaters reached. For our early post-Katrina data, a renovated house exhibited the lowest LPS (0.0 nmols/mg dust), the lowest MA (1.7 ng/mg dust), the lowest Erg (0.3 ng/mg dust) and the lowest Bioactivity-LAL (<5 EU/mL). However, the tile flooring was 'at-risk,' possibly due to contractor activity throughout the home and the lack of an operating ventilation system. A non-renovated home had a 'wet' rug and concrete slab which felt slightly damp, and the home still had visible mold and water damage; however, none of the seven mycotoxins were found in the dust sample. The most VER was found in a non-renovated house (1761 pg/g dust), which did not flood, but sustained extensive roof damage. The house also had visible mold and dark brown water damage stains on the walls and ceilings.

In the 10 dust samples analyzed for mycotoxins, fungal and bacterial markers,

Aspergillus versicolor and *Stachybotrys chartarum*, neither of the fungal species was

found. Natural degradation of fungi can occur over time, even when the metabolites

persist. Also, other fungal species that may potentially produce mycotoxins may explain

the detection of the mycotoxins in the absence of the two fungal species (Tuomi et al

2000). *Aspergillus nidulans* and *Myrothecium verrucaria* may produce sterigmatocystin

and verrucarol, respectively (Jarvis et al 1984; Yu and Leonard 1995).

Reported respiratory allergies and exacerbation due to mold exposure were not prevalent

post-Katrina. 33% of the participants had either a diagnosed or an undiagnosed

respiratory allergy, such as hay fever. None of the participants indicated atopy (genetic

predisposition) for mold and other respiratory allergens. Only one participant, RH5, had a

deceased father with doctor-diagnosed asthma; however, the homeowner did not report

experiencing any respiratory distress pre- or post-storm. Most of those with respiratory

allergies resided in homes that flooded during Hurricane Katrina and had been renovated

at the time of sampling. This was inconsistent with the preliminary findings of a 5-year

long study of New Orleans remediation workers and lung function decline after microbial

exposure; the prevalence of new-onset asthma was elevated among the workers and may

have correlated with the number of hours spent working around the dusty conditions

(Rando Guest Lecture). The only asthmatic participant in our study post-Katrina, RH3,

had doctor-diagnosed allergy-induced asthma and needed to use an inhaler infrequently.

The participant also had the sole positive mold-specific IgE test, which supported the

clinical diagnosis. Otherwise, a single positive specific IgE test may have been

insufficient to confirm mold atopy (Bardana 2003; Bush 2008; Gergen et al 2009). For the 7 participants with elevated total IgE and negative specific IgE, the results indicate that they may be at high risk of sensitization to an allergen or had an infection at the time of sampling, which may or may not have been fungal in nature (Phadia 2006; Sonora Quest 2009). Elevated total IgE levels were not indicative of participation in home-gutting activities.

Respiratory distress symptoms pre- and post-Katrina were reported less often by those participants who had visible mold and/or water damage in their homes, suggesting that the respiratory complications noted may have been either due to mold exposures outside of the home or in conjunction with other environmental factors. All respiratory allergies reported were present before the storm, and only three participants with respiratory allergies reported exacerbation of symptoms, e.g. sneezing and wheezing, post-Katrina. All three participated in post-storm cleanup on some level, and two reported staying in a FEMA trailer for at least a year after the storm. Pre-Katrina, all of the participants were confirmed asthmatic patients, all had breathing problems after physical exertion, and 75% of them required daily use of inhalers or nebulizers. For post-Katrina microbial marker exposures, one person reported experiencing lung trouble that was not present pre-Katrina; however, the participant declined to elaborate on what the health complications were and whether the condition was diagnosed by a doctor. A post-Katrina study determined that although the environmental mold spore counts were relatively high, the lack of a greater health impact from mold may be (in part) due to underreported cases and misidentification as seasonal cold and flu (Barbeau et al 2010).

Overall, mold antibody levels did not correlate with mold exposure duration and severity. Chi square exact tests and Spearman exact correlations were not statistically significant for mold exposure, respiratory health, mask usage or Indoor/Outdoor airborne mold spores, based on total IgE levels for all participants. Eight blood sera had elevated total IgE levels, which were for the following: 1) 2 Control homeowners, with very low indoor and outdoor mold spore counts; 2) the drop-out control homeowner/renter whose environmental conditions were unknown; 3) 2 renovated homeowners, one of whom had doctor-diagnosed allergy-induced asthma, the sole positive mold-specific IgE and very low indoor and outdoor mold spore counts; 4) one non-renovated homeowner of a home with mold levels exceeding 36,000 spores/m^3; however, the homeowner did not conduct any remediation, and the other owner of the home who spent more time in the moldy home did not have elevated total IgE.; 5) a laborer pre-and post-gutting of the non-renovated/partially renovated home, which had mold spore levels exceeding 60,000 spores/m^3, and the laborer reported wearing an N95 mask at all times during the cleanup; and 6) the sole volunteer, whose environmental conditions were also unknown. *Cladosporium* and *Penicillium/Aspergillus* were the primary genera found in the homes, regardless of flood status.

The renovated homeowner had positive specific IgE responses to 8 molds, excluding only *Aspergillus niger* and *Chaetomium globosum*; *Alternaria*, which elicited the strongest response, was not present in the indoor or outdoor air or in the dust sample. The duration and severity of mold exposure was determined by the responses to questionnaires regarding the following: total number of homes gutted after Hurricane Katrina, the

condition of the homeowner's residence and current occupancy status, the level of each homeowner's involvement in home gutting and repair and the use of an N95 mask upon home re-entry and gutting. Only one participant had doctor-diagnosed allergy-induced asthma and required occasional use of asthma medication. Specific IgE testing with ImmunoCAP confirmed the participant's mold allergies.

The main limitations for the research were the small sample size and lack of repeat sample collection, with the exception of the laborers and non/partially renovated home post-Katrina. However, budgetary constraints severely limited sample collection and laboratory analysis capabilities. An attempt to secure additional funding, through the submission of an NIH Challenge Grant, was rejected in April 2009. We included as many similarities in data collection and analysis pre- and post-Katrina as deemed feasible. Due to the limited number of facilities available post-storm to treat asthmatics, the focus was not on specifically targeting an expectedly smaller group. Also, we wanted our post-Katrina data to be more applicable to a wider range of residents.

Air-O-Cell cassettes were used to collect non-viable mold spores, and questionnaires were administered to a majority of the participants (only one exception). Comparisons between the pre- and post-Katrina indoor Air-O-Cell data were difficult to make due to different sample sizes (n = 7 and 26, respectively) and non-repetitive home samples. 10 residual dust samples were analyzed by separate labs for two fungal species commonly associated with the seven mycotoxins of interest.

If future funding for New Orleans household dust analyses becomes available, archived and newly collected dust samples may be analyzed using MSQPCR, and the fungal burden in New Orleans' dust may be compared with other geographical locations using ERMI. Recall bias is often a concern in studies that involve questionnaire data, especially when the information is collected years post-event. We sought to address the issue by incorporating the environmental sampling and blood sera collection into the post-Katrina research. However, it was not possible to completely eliminate confounding variables that may interfere with questionnaire data interpretation, other household allergens and non-residential mold exposures. There are still myriad variables which hinder the ability to fully elucidate the potential association between fungal and bacterial markers, mold exposure and negative health effects. Synergy among cofactors, such as the presence of household dust mites, cockroaches, human and pet dander and pollen, appears to exacerbate asthma and allergies in some exposed persons. It is critical for researchers to gain more knowledge concerning asthma and allergens, as well as understand how to identify and protect vulnerable subpopulations.

X. Conclusions and Recommendations

Overall, the airborne microbial concentrations pre-Katrina (mean I/O = 0.19) were different from post-Katrina's renovated and non-renovated homes (mean I/O = 2.74), although the post-Katrina levels in non-damaged homes (mean I/O = 0.11) were similar to pre-Katrina levels. Compositions of air were similar pre-Katrina and post-Katrina, given that basidiospores, ascospores and *Penicillium/Aspergillus* were predominantly found in both environments. However, pre-Katrina air samples did not yield any Stachybotrys or other water-indicator fungi, and *Cladosporium* was more prevalent post-Katrina than in pre-Katrina samples. The BioCassette was shown to potentially be as efficient at culturable mold spore recovery as the Andersen air sampler (Mann Whitney U = 137; p = 0.22). Dust from non-renovated homes had stronger correlations between microbial marker levels and home characteristics than partially renovated and renovated homes. Pre- and post-Katrina, symptoms of respiratory distress were less likely to be reported by those residing or working in moldy buildings. Post-Katrina, those who participated in home-gutting were less likely to have elevated total IgE. Mold antibody levels did not correlate with mold exposure duration and severity.

To the authors' knowledge, there have been no Hurricane Katrina-related studies published which examine the blood sera, air and dust samples of interviewed remediation workers and residents in New Orleans, LA. In addition, there have been no data available from residential mold exposure studies conducted before Hurricane Katrina. In light of increased natural disasters and recent household flooding nationwide, this study provides pertinent information for assessing potential inhalation mold exposures and mold-specific

antibody responses in exposed residents and remediation workers. More studies regarding

residential microbial markers immediately after catastrophic natural disasters are needed

to further elucidate potential exposures in returning residents and workers.

XI. References

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X. Appendices

This work is dedicated in honor of my husband, John Lewis Jr. and MAC's Layla Marie. It is dedicated in memory of the following: Ruthie Truss, MAC's Leonidas Maximus, John and Marie Lewis.

The photo (A Snowy Day in New Orleans, 12-11-2008) is the property of Jocelyn Lewis.

"It is not the critic who counts; not the man who points out how the strong man stumbles, or where the doer of deeds could have done them better. The credit belongs to the man who is actually in the arena, whose face is marred by dust and sweat and blood; who strives valiantly; who errs, who comes short again and again, because there is no effort without error and shortcoming; but who does actually strive to do the deeds; who knows great enthusiasms, the great devotions; who spends himself in a worthy cause; who at the best knows in the end the triumph of high achievement, and who at the worst, if he fails, at least fails while daring greatly, so that his place shall never be with those cold and timid souls who neither know victory nor defeat." -- Theodore Roosevelt (speech at the Sorbonne, Paris in 1910) located at http://www.theodoreroosevelt.org/life/quotes.htm.

Don't aim for success if you want it; just do what you love and believe in, and it will come naturally. -- David Frost; at http://www.brainyquote.com/quotes/quotes/d/davidfrost107983.html.

Appendix A. Three Submitted manuscripts

Manuscript 1: "Comparison and characterization of mold spore collection efficiency of BioCassette™ and Andersen Sampler and mold exposure assessment in New Orleans"

Manuscript 2: "Analysis of fungal and bacterial markers in water-damaged New Orleans homes post-Hurricane Katrina"

Manuscript 3: "Fungal Allergens: An Investigation of biomarkers of mold exposure in New Orleans, Louisiana"

Title:
Comparison and characterization of mold spore collection efficiency of BioCassette[TM]
and Andersen Sampler and mold exposure assessment in New Orleans

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Running Title: "BioCassette vs. Andersen"

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Abstract

Objectives: To compare fungal collection efficiency of BioCassette™ and Single Stage N6 Andersen Cascade Impactor, to characterize airborne mold in New Orleans homes (2004) and to assess mold exposure.

Andersen, BioCassette and Air-O-Cell were tested. Differences in collection efficiencies were not statistically significant (Mann-Whitney, p = 0.22). BioCassette may recover mold as efficiently as Andersen. Basidiospores, *Cladosporium*, Non-sporulating colonies and *Penicillium* were predominant. Mold levels did not correlate with asthmatics' allergen exposure questionnaire responses.

Keywords: Air-O-Cell, Andersen, BioCassette, airborne mold, New Orleans

Highlights of the study:

- BioCassette™ may recover mold as efficiently as Andersen impactor.
- Viable: *Cladosporium, Penicillium* and non-sporulating colonies
- Non-viable: basidiospores, *Penicillium/Aspergillus* and ascospores
- Highest device & species mean I/O ratios: BioCassette (1.09) and Curvularia (6.42)
- Mold levels did not correlate with asthmatics' allergen exposure questionnaires.

Funding Source

1. Introduction[1]

Potential exposure to mold remains an integral concern in residential indoor air quality, especially for locations housing immunocompromised individuals. A study by Salvaggio et al found elevated ambient mold spore levels in New Orleans from March until November, resulting in increased hospitalization of asthmatics (1971). There are currently no federal mold standards or regulations; however, the public's perception and the current scientific literature indicate the need for continued indoor mold sampling (Gots et al 2003). Concern exists for exposure to viable mold spores and to potentially allergenic non-viable mold spores and hyphal fragments (Niemeier et al 2006).

In 2003, Environmental Microbiology Laboratory, Inc. (EMLab) released a culturable mold air sampling device, the BioCassette™ (BC). At the time, the Single Stage N6 Andersen Cascade Impactor ("Andersen") was the "gold standard" in sampling for viable mold in IAQ studies (Thermo Scientific 2009). However, the newer device was marketed as having several advantages over the Andersen, including: 1) it's a single-use device pre-filled with either Malt Extract Agar (MEA) or Tryptic Soy Agar (TSA), no field-cleaning of the device required and no cross contamination, and 2) it's compact, lightweight and potentially more cost-effective than bulkier Andersen (EMLab 2010).

In the present study, the objectives were to compare the fungal collection efficiency of the BC and the Andersen and to characterize airborne mold and allergen exposure in New Orleans homes. Other studies have compared the collection efficiency of the Andersen with samplers such as Reuter Centrifugal Air, Surface Air Systems, mini-Burkard and viable Air-O-Cell cassettes (Godish et al 2008; Lee et al 2004). To the authors'

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[1] Abbreviations: Single Stage N6 Andersen Cascade Impactor (Andersen); BioCassette™ (BC); Air-O-Cell Cassette (AOC)

knowledge, this is the only non-internal study to-date that evaluates the collection efficiency of the BC versus the Andersen. The study also provides pre-Hurricane Katrina data regarding allergen exposure for asthmatics in New Orleans.

2. Methods

2.1. Site Selection

The master bedroom and living room in three homes in New Orleans, LA, were sampled in late July 2004, based on the presence of at least one asthmatic resident. Two adults and two children, ages 11- and 16 years old, answered questions regarding home flooding, visible mold or moisture, pet ownership, air conditioning and asthma symptoms. An additional bedroom was sampled in House 3, which housed both an asthmatic adult and child.

2.2. Air Sampling

In each home, a Single Stage N6 Andersen (Thermo Fisher Scientific, Franklin, MA) and a BioCassette (EMLab, Phoenix, AZ) loaded with 2% MEA were run side-by-side simultaneously. In all three homes, the devices were operated for one, two and three minute-intervals to collect 28.3L, 56.6L and 84.9L of air, respectively. Outdoor air samples were collected for one minute at each front door. A total of 16 living room, 24 bedroom and 6 outdoor culturable mold samples were collected. An Air-O-Cell cassette (Zefon International, Ocala, FL) was used in each sampling room and outdoors for five consecutive minutes at 15 L/min. to measure non-viable fungal spores in 10 samples. BC and Andersen samples were shipped to EMLab (California) for culturing and analysis of

colonies, and Air-O-Cell cassettes were shipped to EMLab P&K (New Jersey) for analysis of fungal spores and hyphal fragments.

3. Results and Discussion

SPSS was used for all statistical analyses (IBM SPSS Statistics, vers.19.0, Somers, NY). All data were determined to be non-normally distributed based on Quantile-Quantile plots, and statistical significance was determined at the 5% level.

3.1 Questionnaires

None of the homes had ever flooded. All 3 houses had leaks during sampling, but only Houses 1 and 3 had visible mold. House 2 contained a pet (bird). Houses 1 and 2 had plants and window air conditioning units; Houses 3 had central air. No humidifiers or dehumidifiers were used. Houses 2 and 3 had both smokers and asthmatic children in residence (including grandchildren), although House 1 had neither. House 1 and 2 residents were hospitalized twice in the prior 12 months for breathing problems. All four participants had breathing problems after physical exertion, and only the adult in House 3 rarely needed to use a nebulizer or inhaler. The other three participants required at least daily use of asthma medication. No one had skin testing conducted for common allergens.

Questionnaire data were categorized according to increased risk of asthma exacerbation and compared with mean I/O colony and spore counts. They included: mold growth (leaks and/or visible mold), pet ownership, air conditioning (window or central), environmental tobacco smoke, respiratory health (frequency of medication use) and age (child under 18 years of age or adult) (Cummings et al 2008). Exact chi square tests and

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Spearman correlations calculated for all categories, based on age, mean I/O colonies and mean I/O spore counts, determined that there were no statistically significant differences.

3.2 Culturable and non-culturable sampling

Results for 1-minute and total indoor and outdoor BC, Andersen and AC sampling are provided in Table A1. For the AOC cassette sampling, no fungal hyphal fragments were found. However, the most spores per cubic meter were evident in House 2. BC consistently exhibited the most CFUs/m^3. Overall, the highest CFUs/m^3 were found in House 3, even when considering each bedroom and the living room separately. The house had small patches of mold in Bedroom 1 and in the bathroom near Bedroom 2. Although there are no mold standards, the levels of culturable mold in the home fell outside of the recommended ACGIH indoor fungal guidelines of no more than 1000 CFU/m^3 (Jo et al 2005).

EMLab also field-tested the BioCassette against the Andersen to compare sampler collection efficiencies. Fifty pairs of side-by-side samples were collected simultaneously for four minutes each in five outdoor locations in San Diego, CA; no statistically significant difference was found in the collection efficiency of the culturable air samplers (Purves and Georgianna 2003). We found no statistically significant difference between the BC and Andersen (Mann-Whitney U: 137.0; p = 0.22); therefore, it was concluded that the BioCassette may recover culturable mold as efficiently as the Andersen.

3.2 Characterization of Fungi

In Table A.2, overall and species-specific mean I/O Ratios and indoor percentages of total colonies and counts were calculated for each device (Microsoft Office 2010, Excel, Redmond, WA). The predominant fungal species cultured, in descending order, were: *Penicillium*, Non-sporulating colonies, *Cladosporium* and *Curvularia*. The remaining 12% of the colony counts for BC and 19% for Andersen were comprised of *Bipolaris/Drechslera*, *Fusarium* and *Mucor* in the 1-minute samples. Primary fungal species cultured outdoors were: non-sporulating colonies, Cladosporium, Penicillium and Curvularia. In the full 6-minute sampling time frame, trace amounts of *Alternaria*, *Aspergillus fumigatus*, *A. nidulans*, *A. niger*, *A. ochraceous*, *A. versicolor*, *Aureobasidium pullulans*, *Epicoccum*, *Paecilomyces*, *Syncephalastrum* and *Verticillium* were found both indoors and outdoors.

Non-viable spores primarily were: basidiospores, *Penicillium/Aspergillus*, ascospores, *Curvularia* and *Cladosporium*. The remaining 5% of the spores were: *Bipolaris/Drechslera* group, *Epicoccum*, *Ganoderma*, *Nigrospora*, *Pithomyces*, *Smuts/Periconia/Myxomycetes*, *Stemphylium* and *Zygosporium*. It is not unusual for basidiospores and ascospores to be detected in the air, yet remain unlikely to form colonies on MEA (Lee et al 2006). Statistically significant Spearman correlations were not observed for non-viable spores and their species-specific viable colony counterparts, unlike the results found in another study (DeKoster et al 1995). Our results illustrate Zefon's internal study's recommendation against comparing non-viable Air-O-Cell data with those collected by viable samplers (Tsai et al 1998).

3.4 Conclusions

In conclusion, it was determined that the BioCassette may recover airborne mold as efficiently as the Single Stage N6 Andersen Cascade Impactor. However, the main limitation is the very small sample size of three houses. Overall, the primary mold species cultured with the BioCassette™ and Andersen were among the main spore generators in the non-viable Air-O-Cell cassette sampling. Questionnaire data, regarding home characteristics, mold exposure and asthma symptoms, did not yield any statistically significant results for exact chi square and spearman correlations based on age, mold colonies or mold spore levels.

Acknowledgments

Disclosure Statement

The authors have no potential or actual conflicts of interest which may bias this work.

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Appendix

Table A.1. Combined Indoor and Outdoor Sampling for BioCassette, Andersen and Air-O-Cell Cassettes

	BCIn1	BCTotal	BCOut	AndersenIn1	AndersenTotal	AndersenOut	AOCIndoor	AOCOutdoor
House 1	352	579	160	246	411	89	820	6700
House 2	458	991	355	388	857	158	2450	14000
House 3	2840	5629	836	1517	4209	1448	2010	7000
Total	3650	7199	1351	2151	5477	1695	5280	27700

[a]. BioCassette (BC) and Andersen indoor and outdoor sampling times are for 1-minute intervals (n = 8 total), and all Air-O-Cell cassette (AOC) samplings are for 5 minutes (n = 7).

[b]. Total indoor sampling (BCTotal and AndersenTotal) = overall culturable mold level in bedrooms and living rooms sampled over the full 6 minutes (n = 40).

[c]. Units for BC and Andersen are in Colony-forming units (CFU) per cubic meter, and AOC units are in spores per cubic meter.

Table A.2. Mean Indoor/Outdoor Mold Spore Ratios and Indoor Fungal Taxa Percentages

Sampling Device	Overall	Ascospores	Basidiospores	*Cladosporium*	*Curvularia*	Non-sporulating	*Penicillium*
BioCassette	1.09	n/a	n/a	1.45 (17)	n/a	1.06 (18)	5.29 (53)
Andersen	0.88	n/a	n/a	1.24 (15)	6.42 (5)	0.48 (23)	2.66 (38)
Air-O-Cell	0.08	0.03 (6)	0.06 (60)	0.02 (1)	0.42 (6)	n/a	0.10 (22)

[d.] Numbers preceding parentheses indicate Mean Indoor/Outdoor (I/O) ratios; parenthetical numbers are percentages of total indoor colonies or counts by species.

[e.] All ratios given for BioCassette and Andersen include only the combined indoor and outdoor sampling for 1-minute intervals.

[f.] *Penicillium* category includes *Aspergillus* spores for Air-O-Cell cassette sampling.

Title:
Fungal and bacterial markers in previously water-damaged New Orleans homes post-Hurricane Katrina

Running Title:
Microbial markers in New Orleans house dust

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ABSTRACT

The study's objective was to establish if significant correlations existed between fungal and bacterial markers in dust from previously water-damaged New Orleans homes in different stages of repair and home characteristics.

Gas chromatography-tandem mass spectrometry (GC-MSMS) analyzed *Stachybotrys* mycotoxins (trichodermol, verrucarol), ergosterol, muramic acid and lipopolysaccharide (using 3-hydroxy fatty acids with 10 to 18 carbon chains as markers). Endotoxin biological activity was measured with Limulus amebocyte lysate assay (LAL). High performance liquid chromatography-tandem mass spectrometry (HPLC-MSMS) analyzed *Aspergillus* mycotoxins (aflatoxin B1, gliotoxin and sterigmatocystin) and *Stachybotrys* macrocylic trichothecenes, satratoxins G and H. Home characteristics were: building style and frame, chemical treatment, flood status, floor type and moisture, occupancy, relative humidity, remediation status, temperature and visible mold and/or water damage.

Non-renovated homes had higher levels of muramic acid and lipopolysaccharides (C:12 – C:18, especially C:16) and lower temperatures and relative humidity levels. Biological activity of gram-negative bacteria and ergosterol only weakly correlated with remediation status. Neither *Aspergillus versicolor* nor *Stachybotrys chartarum* was detected in of the 10 dust samples analyzed with ELISA for antigen, although two mycotoxins predominantly produced by these species, sterigmatocystin and verrucarol, respectively were present in varying levels. The use of a chemical treatment did not correlate with microbial markers. More studies, regarding microbial markers in dust after catastrophic natural disasters, are needed to further elucidate potential exposures for returning residents and workers.

Keywords: endotoxin; fungi; mass spectrometry; mycotoxins; New Orleans; water

damage

Highlights:

- Non-renovated homes had higher levels of muramic acid and lipopolysaccharides.

- Endotoxin bioactivity and ergosterol weakly correlated with remediation status.

- Neither *Aspergillus versicolor* nor *Stachybotrys chartarum* antigen was detected.

- Sterigmatocystin and verrucarol, *A. versicolor* and *S. chartarum* mycotoxins, found.

- Chemical treatment did not correlate with microbial marker levels.

1.1. Introduction

After Hurricane Katrina landed in the Gulf Coast region in late August 2005, more than 100,000 of New Orleans' households, approximately 50%, were inundated with 2 feet or more of floodwater (HUD 2005). For several weeks, these buildings festered in pools of stagnant water, ultimately resulting in large swaths of mold-infested buildings. The nutrient-rich organic matter and high moisture levels within the buildings' walls, furnishings and other water-logged materials were prime breeding grounds for indoor fungi (HUD 2006); some of these fungi were already ubiquitous in outdoor areas of New Orleans, especially due to the relatively high temperatures and humidity throughout the area. In 2006, the Centers for Disease Control (CDC) determined that 51 of the 112 New Orleans area homes surveyed exhibited visible mold indoors (CDC 2006).

Mold and bacterial components may exacerbate asthma and cause other immunological reactions (Hyvärinen et al 2006; Maier et al 2010; Poole et al 2010; Sahakian et al 2008; Saraf et al 1997; Sebastian et al 2005; Sullivan et al 2001). However, there are currently no exposure standards for inhalation of molds, mycotoxins or endotoxins (Robbins et al 2000; Poole et al 2010). Ergosterol (Erg), a component of fungal cell membranes used as a surrogate for fungal biomass, has been associated with damp buildings and structural materials (Sullivan et al 2001). Muramic acid, a component of peptidoglycan associated only with gram-positive and -negative bacteria, represents largely gram-positive bacteria, which contain more MA than gram-negative (Nilsson et al 2004; Sebastian et al 2004; UWM 2006). Endotoxins may synergize with molds and mycotoxins and increase health risks (Hirvonen et al 2005; Thrasher et al 2009). Airborne endotoxins may eventually

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settle in floor dust and re-aerosolize upon disturbance, which can become inhaled or ingested (Adhikari et al, 2010; Butte et al 2002).

The primary objective of this study was to determine if significant correlations exist between fungal and bacterial markers in dust samples collected from previously water-damaged homes and characteristics of the homes, such as remediation status, building style and frame, use of chemical treatment, visible mold and/or water damage, flood status and type and moisture content of flooring material in the sampling room. Four mycotoxins (toxic fungal secondary metabolites) that are primarily produced by *Stachybotrys chartarum* were studied, including verrucarol (VER), trichodermol (TRID) and the macrocyclic trichothecenes, satratoxin G and satratoxin H (SATG/H); VER is also a hydrolysis product of such trichothecenes. Aflatoxin b1 (AFLAB1), gliotoxin (GLIO) and sterigmatocystin (STRG), which are primarily produced by *Aspergillus* species, were also studied (Bennett et al 2003; Bloom et al 2007; Bloom et al 2009a; Bloom et al 2009b). Ergosterol (Erg) for fungal biomass, muramic acid (MA) to represent (mainly gram-positive) bacteria, lipopolysaccharide (LPS) for the chemical aspect of endotoxins in gram-negative bacteria (comprised of 3-hydroxy fatty acid carbon atoms, including C:10 to C:18) and the Limulus amebocyte lysate (LAL) assay to determine the biological activity of endotoxins, were the additional parameters tested (Sullivan et al 2001). High performance liquid chromatography-tandem mass spectrometry (HPLC-MSMS) was used to analyze STRG, AFLAB1, GLIO and SATG/H. Gas chromatography-tandem mass spectrometry (GC-MSMS) was used to measure the following: VER, TRID, Erg, MA and LPS.

2.1. Materials and Methods

2.1.1. Sampling Site Selection

Fifteen previously water-damaged single-family New Orleans homes were sampled during the winter months, from November 2007 to February 2008, approximately 26 to 29 months after the recession of the floodwaters. These water-damaged homes were characterized according to remediation status, including 6 renovated, 5 partially renovated and 4 non-renovated homes (Table A.1). Remediation status was defined as the following: 1) renovated sites had new sheetrock and flooring; 2) partially renovated sites were gutted (e.g. damaged sheetrock and/or flooring removed, but mold or water-damage still visible and 3) non-renovated sites were ungutted, with original damaged sheetrock and flooring intact. A bulk dust sample was collected with a small broom and dustpan from the gutted (partially renovated) living room in a split-level home with a non-renovated upper level for same-house comparison.

2.1.2. Dust Collection:

The flooring material at the testing sites consisted of wood, concrete slab, carpet, tile, a small rug on slab or linoleum. Before collecting the dust samples, the moisture level in the flooring was measured using a GE® Protimeter MMS Plus (General Electric Company Measurement and Control Solutions, CA) to determine materials that were 'dry,' 'at risk' or 'wet.' In each house, samples were collected from one room with considerable mold growth or water damage. If neither sign of biological contamination was evident at the time of sampling, the designated sampling site was chosen using the homeowner's recollection of prior moldy or water-damaged sites pre-renovation.

Dust samples were collected from a $1m^2$ area in the center of the floor in each home for 5 minutes, following the Housing and Urban Development (HUD) Dust Sample Collection Protocol; a Filter Queen Majestic vacuum cleaner (Health-Mor Industries, OH) and small single-use filters (EMLab P&K, Phoenix, AZ) were used to collect dust, which were secured in small, zipper-closure plastic bags pre-labeled with each home's study ID (Ashley, 2006). Due to the heavy sediment caked onto the hard floors and the very dusty conditions in 4 of the homes, 4 dust samples (3 partially renovated and 1 non-renovated home) were collected using a small dustpan and an accompanying plastic-bristled broom instead of a vacuum (Bloom et al 2009a). In a single home, one vacuum sample was collected in the carpeted master bedroom upstairs from a $1m^2$ area in the center of the room. An additional broom and dustpan bulk sample was obtained from a 1m2 area in the center of the wood flooring in the gutted dining room downstairs. To avoid cross-contamination of samples, the dustpan and the broom were washed with a mild detergent and water, sprayed with 70% ethanol and allowed to fully dry after using at each home. In the field, collected samples were placed in a specimen cooler containing frozen icepacks before storage at -20°C in the lab. Post collection, all dust samples were shipped to the University of Lund in Sweden for analysis of the microbial markers.

2.1.3. Dust analysis.

The dust samples were homogenized and divided into portions for the different analyses. Erg, MA, and LPS were determined by GC-MSMS using an ion-trap instrument as described previously (Sebastian et al 2004). In brief, aliquots of the dust samples were

subjected to acid (LPS, MA) respectively alkaline (Erg) hydrolysis, purified, and derivatized prior to analysis; LPS was calculated by summarizing the number of moles of the found 3-hydroxy acids and dividing by four (Sebastian et al 2004). Separate dust aliquots were extracted with methanol; after purification, a portion of each extract was analyzed by HPLC-MSMS, using a triple quadrupole instrument, for SATG, SATH, GLIO, AFLAB1, and STRG, whereas a remaining portion of the methanolic extract was derivatized and analyzed by triple quadrupole GC-MSMS for VER and TRID (Bloom et al 2009a). Finally, separate aliquots of the dust samples were extracted with a buffer and subjected to analysis for endotoxin bioactivity using a chromogenic Limulus method (Mårtensson et al 1997).

Dust samples from 10 homes (all 6 partially renovated samples and samples from 3 non-renovated and 1 renovated homes) were also sent to a third-party laboratory (INDOOR Biotechnologies, Charlottesville, VA) in the winter of 2009 for Enzyme-linked Immunoassays (ELISA) for *Aspergillus versicolor* and *Stachybotrys chartarum*. The dust was sieved through a Number 45 mesh screen to remove larger particulates and fibers; 100 mg of fine dust was extracted with 2.0 mL PTS-T, 0.05% buffer and analyzed from a 1:2 dilution (Dr. Bryan Smith, personal communication).

2.1.4. Statistical Analyses.

All statistical analyses were conducted with SPSS ((IBM SPSS Statistics software, Version 19, Somers, NY). Statistical testing for all hypotheses was conducted at the 10% level of significance. The markers and home characteristics were not normally distributed, as determined by Q-Q plots. Descriptive statistics, including mean, median

and interquartile ranges, were determined for the biomarkers in a modified version of a similar study (Sordillo et al 2011). Kruskal Wallis tests with exact significance were conducted for microbial markers and their ratios, based on remediation status. P-values greater than 0.10 and confidence intervals containing '1', for respective tests, were not statistically significant. Chi square exact tests were calculated to determine proportions of home characteristics attributed to remediation status. Spearman correlations (a nonparametric statistical method) were calculated for all variables to measure associations and presented in a modified form of two previous studies (Lee et al 2006; Sordillo et al 2011). An interpretation of high correlation was designated as correlation coefficients from 0.7 to 0.9, moderate correlations were from 0.5 to 0.7 and low correlations were from 0.3 to 0.5 (Calkins 2005). Weak statistically significant data had p-values greater than 0.05, but less than 0.10; strong significance indicated a p-value less than 0.05.

3.1. Results and Discussion

3.1.1. Bacterial components and Erg

Potential associations between home characteristics and levels of mold and bacteria have been observed in the literature (Adhikari et al 2010; Kostamo et al 2005; Park et al 2004). Gram-negative bacteria are detected by measuring the levels of two components of endotoxins: the chemical marker, LPS, in gram-negative bacteria's cell membranes and the biological activity using LAL (Saraf et al 2009). LPS is measured with GC-MSMS by analyzing dust for 3-hydroxy fatty acids (3-OHFAs), including shorter carbon chains from C:10 to longer C:18 chains. 3-OHFAs from C:10 to C:14 may reflect actual

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endotoxin levels, whereas longer chain 3-OHFAs (C:16 – C:18) may indicate the presence of some Actinobacteria (Sebastian et al 2005). In our study, houses with carpet and wood had higher levels of C:16 and C:18, regardless of flood status, indicating that Actinobacteria may have been present on at least 44% of the floors sampled. Stronger correlations were observed between C:12, C:14 and LAL (rho = 0.781 and 0.772, respectively; p < 0.001) than between C:16, C:18 and LAL (rho= 0.700 and 0.707, respectively; p < 0.5), as in other studies (Park et al 2004; Szponar et al 2000).

Chi square exact tests for home characteristics, based on remediation status, are presented in Table A.2. Statistically significant differences (p < 0.10) for renovated, partially renovated and non-renovated homes were seen for floor moisture readings, flooring type and the presence of visible mold and/or water damage. Most of the renovated homes were single-story, brick-framed buildings, whereas the partially and non-renovated homes tended to be split-level, wooden-frame residences. All renovated homes and 75% of the non-renovated homes were chemically treated (with either bleach and water or a chemical unbeknownst to the homeowner), and only 33% of the partially renovated homes had undergone chemical treatment. For the partially renovated homes, 33% also had signs of water-damage still present, but no visible mold growth. Temperature and humidity levels were predominantly higher in partially renovated homes than for renovated and non-renovated homes.

Descriptive statistics and Kruskal Wallis tests, based on remediation status, for the fungal and bacterial components are presented in Table A.3. Kruskal Wallis tests were statistically significant (p-values all < 0.05) only for LPS, C:12 – C:18 and MA. Microbial marker median ratios and 95% confidence intervals are presented in Table A.4.

Statistically significant differences, inter- and intra-group based on remediation status, were observed for Erg/LPS, MA/LAL and MA/LPS. For Erg/LAL, significant differences were seen overall and for renovated and non-renovated groups. For Erg/MA, only differences within the non-renovated group were statistically significant.

Statistically significant Spearman correlation coefficients are shown in Table 5. Highly strong correlations were observed between visible damage and MA, LPS and C:12 – C:18 and between remediation status, C:12 – C:18 and MA. Renovated homes were associated with lower levels of MA and C:12 - C:18. Non-renovated homes generally had higher levels of MA and 'dry' floors, although there was a low weak correlation between MA and floor moisture readings. Moderately strong correlations were observed between visible mold and/or water damage and the following: LPS, C:12 - C:18 and the use of a chemical treatment. Non-renovated homes were associated with lack of treatment. Erg weakly correlated with visible mold and water-damage in partially renovated and non-renovated homes; some renovated homes also had detectable levels of Erg. This finding was consistent with other studies because LPS, Erg and MA naturally occur in all house dusts, not exclusively in water-damaged, unrepaired homes (Sebastian et al 2003; Sebastian et al 2005). It has been noted that increased levels of MA and some endotoxins may have protective effects for some asthmatic children when exposed early in life (Park et al 2001; Sordillo et al 2010; Sordillo et al 2011; Zhao et al 2008).

LAL weakly correlated with wooden frames, occupied homes, flooring type (e.g. carpet and concrete had lower levels) and non-renovated homes, although some renovated and partially-renovated homes actually had higher levels than non-renovated homes. This may have been attributable to higher levels of remediation activity in these

homes, which may have aerosolized bacteria and resulted in a lower levels in dust compared to non-renovated homes; the air in the homes were not tested for culturable bacteria.

3.1.2. Mycotoxins and mold species

The most common fungi found in post-Hurricane Katrina studies were: *Aspergillus/Penicillium* species, *Paecilomyces, Trichoderma* and *Stachybotrys*, and airborne endotoxins were also observed (Chew et al 2006; Riggs 2008; Rao et al 2008; Schwab et al 2007; Solomon et al 2006). *Stachybotrys* is the predominant producer of VER, TRID and macrocyclic trichothecenes, such as SATG and SATH (Bennett et al 2003; Bloom et al 2009a). *Aspergillus* species, particularly *A. flavus, A. fumigatus* and *A. versicolor*, are often the culprits in the production of AFLAB1, GLIO and the aflatoxin precursor, STRG, respectively (Klich 2007; Sulllivan et al 2001). Aflatoxin B1, the most researched strain compared to B2, G1 and G2, has been designated by the International Agency for Research on Cancer (IARC) as a Group I human carcinogen (Bennett et al 2003; IARC 1993; Miliţă et al 2010). We did not detect any SATG/H or GLIO in any of the dust analytes.

A similar study on mycotoxins in New Orleans was conducted by Bloom et al, which detected the presence of VER from 600 to 18000 pg/g and STRG from 16000 to 28000 pg/g (2009a). We found that STRG negatively correlated with occupied homes, as higher levels were predominantly observed in non-renovated homes. We detected the most STRG (7552 pg/g) in a non-renovated home with the highest level of MA (37.3 ng/mg), the only detected level of AFLAB1 (0.2 pg/g), the second highest LAL (170

EU/mg) and a 'dry' wooden floor. The second highest level of STRG (3303 pg/g) was in

a partially renovated house with the most Erg (22.6 ng/mg) and an 'at-risk' slab floor.

Both houses flooded, and mold and signs of water damage, e.g. mud on the walls from

the floodwater, were still visible.

TRID and VER were present in 25% and 32% of the samples respectively,

although the median levels of VER (137.6 pg/g) were more than 45 times higher than

TRID (3.4 pg/g). A moderately strong negative correlation was observed between lower

temperatures and higher VER, due to non-renovated homes. The most VER (1761 pg/g)

was found in a carpeted house that did not flood, but did sustain extensive roof damage.

Mold and water stains were evident on the walls and ceilings. The home did have the

second highest level of VER (272.3 pg/g). However, the homeowner reported having a

professional contractor chemically treat the unoccupied home a few weeks before

sampling, although no significant correlations were observed between chemical treatment

and microbial marker levels detected in homes. One home had a 'wet' rug and concrete

slab which felt slightly damp, and the home still had visible mold and water damage;

however, no mycotoxins were found in the dust.

Neither *Aspergillus versicolor* nor *Stachybotrys chartarum* was seen in any of the

10 dust samples analyzed for antigen with ELISA eleven months after the microbial

marker analyses. Natural degradation of fungi can occur over time, even when the

metabolites persist. Also, other fungal species that may potentially produce mycotoxins

may explain the detection of the mycotoxins in the absence of the two fungal species

(Tuomi et al 2000). *Aspergillus nidulans* and *Myrothecium verrucaria* may produce

sterigmatocystin and verrucarol, respectively (Jarvis et al 1984; Yu and Leonard 1995).

4.1. Conclusions

Non-renovated homes had higher levels of MA and LPS (C:12 – C:18, especially C:16) than partially renovated and renovated homes, although the temperatures and relative humidity levels tended to be lower. LAL and Erg only weakly correlated with remediation status. Neither *Aspergillus versicolor* nor *Stachybotrys chartarum* antigen was detected in of the 10 dust samples analyzed with ELISA, although two mycotoxins predominantly produced by these species, STRG and VER, respectively were present in varying levels. The use of a chemical treatment did not correlate with microbial markers. More studies, regarding microbial markers in dust after catastrophic natural disasters, are needed to further elucidate potential exposures for returning residents and workers.

5.1. Acknowledgements

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7.1. Appendix

Table A.1. New Orleans home characteristics (post-Hurricane Katrina)

Classification	Characteristic	Remediation Status[1]		
		Renovated (Controls) (n=6)	Partially Renovated[2] (n =6)	Non-Renovated (n= 4)
Building style	Single-story	4	2	2
	Split-level	2	4	2
Building frame	Brick	4	1	1
	Wood	2	5	3
Chemical Treatment	Untreated	0	4	1
	Treated	6	2	3
Damage	Visible mold	0	4	4
	Water Damage only	0	2	0
Flood Status	Flooded (range in m)	1.3 – 2.3	0.2 – 3.3	1 – 3.3
	Roof Damage only	0	1	1
Floor Moisture Status	Dry	1	4	3
	At-Risk	5	2	0
	Wet	0	0	1
Floor Type	Carpet	0	0	2
	Tile	4	0	0
	Wood	2	2	1
	Other	0	4	1
Residence Status	Not occupied	4	3	4
	Occupied	2	3	0
Thermohygrometry	Relative Humidity (%)	39-74	43-78	37-64
	Temperature (°C)	16-26	16-27	17-24

[1]Renovated= new sheetrock and flooring; Partially Renovated= gutted, but visible damage; Non-Renovated=ungutted
[2]Includes 1 bulk dust sample from gutted living room in a split-level home with non-renovated upper level

Table A.2. Chi square (χ^2) exact tests for home characteristics by remediation status

Characteristic	Chi square statistic	p-value
Building Frame	3.233	0.30
Building Style	1.409	0.81
Flood Status	7.685	0.17
Flooring Moisture	8.151	**0.05**
Flooring Type	15.259	**0.01**
Mold and/or water damage	13.388	**0.00**
Occupancy status	2.559	0.33
Treatment status	4.392	0.16

[1]Significance determined as ($p < 0.10$) (bold)

Table A.3. Descriptive statistics and Kruskal Wallis tests for house dust microbial markers.

Classification	Type	n	Mean	Median	Interquartile Range	Kruskal Wallis[4]
Fungal	Ergosterol (ng/mg) (N=16)	16	9.4	10.0	5.3 – 11.5	χ^2= 3.930 (0.14)
	Mycotoxins (pg/g) (N=16)					--
	Aflatoxin B1(AFLAB1)	1	0.2	0.2	--	--
	Gliotoxin (GLIO)	0	--	--	--	--
	Satratoxins G and H (SAT G/H)	0	--	--	--	--
	Sterigmatocystin (STRG)	8	1359.5	4.6	2.3 – 830.7	χ^2= 0.718 (0.72)
	Trichodermol (TRID)	4	3.4	3.4	0.9 – 5.9	χ^2= 1.650 (0.50)
	Verrucarol (VER)	5	435.2	137.6	3.4 – 272.3	χ^2= 0.022 (0.99)
Bacterial						
Gram Negative	Endotoxin-LAL (EU/mg) (N=16)	16	90.0	80.0	42.5 – 135	χ^2= 4.151 (0.12)
	Endotoxin-LPS (pmols/mg) (N=16)	16	44.0	39.8	22.0 – 54.6	χ^2= 9.647 (0.00)
	C:10	16	7.8	2.9	1.8 – 11.2	χ^2= 3.107 (0.22)
	C:12	16	22.8	16.9	8.9 – 35.6	χ^2= 8.443 (0.01)
	C:14	16	47.7	38.7	16.9 – 57.0	χ^2= 9.283 (0.00)
	C:16	16	55.2	55.4	28.1 – 75.3	χ^2= 10.426 (0.00)
	C:18	16	42.4	31.1	19.6 – 66.5	χ^2= 9.107 (0.00)
Gram Positive	Muramic acid (ng/mg)(N=16)[3]	13	19.7	14.7	6.9 – 34.2	χ^2= 6.945 (0.02)

[1] Abbreviations: Limulus amebocyte lysate (endotoxin-bioactivity); LPS- lipopolysaccharide

[2] '--'= not applicable

[3] Muramic acid - analysis failed in 3 samples

[4] Kruskal Wallis tests based on remediation status = χ^2 (p-value in parentheses); significance: p < 0.05

Table A.4. Microbial marker median ratios by remediation status

Markers	Overall	Renovated (n=6)	Partially renovated (n=6)	Non-renovated (n=4)
Erg/LAL	**0.2 (0.1 – 0.2)**	**0.2 (0.1 – 0.2)**	0.1 (0.0 – 1.0)	**0.2 (0.1 – 0.2)**
Erg/LPS	**0.2 (0.1 – 0.4)**	**0.3 (0.1 – 0.7)**	**0.2 (0.1 – 0.6)**	**0.2 (0.1 – 0.2)**
Erg/MA	0.4 (0.3 – 1.2)	0.8 (0.2 – 1.3)	0.3 (0.3 – 1.5)	**0.4 (0.3 – 0.4)**
LPS/LAL	0.6 (0.3 – 1.0)	0.6 (0.2 – 1.2)	0.4 (0.3 – 1.8)	0.9 (0.7 – 1.4)
MA/LAL	**0.3 (0.1 – 0.5)**	**0.2 (0.1 – 0.5)**	**0.3 (0.1 – 0.9)**	**0.4 (0.2 – 0.5)**
MA/LPS	**0.4 (0.3 – 0.6)**	**0.4 (0.4 – 0.6)**	**0.5 (0.3 – 0.8)**	**0.3 (0.3 – 0.5)**

[1]Abbreviations: Erg-Ergosterol; LAL-*Limulus* amebocyte lysate assay; LPS-lipopolysaccharide; MA-muramic acid
[2]Parenthetical numbers indicate 95% confidence intervals; CI not including '1' is statistically significant (bold).

Table A.5. Spearman correlation coefficients for biomarkers and home characteristics.

Category	Damage	Flood Status	Floor Moisture	Floor Type	House Frame	House Style	Occupancy	RH	RS	Temp.	Treatment
Fungal											
Ergosterol (n=16)	0.449	--	--	--	--	--	--	--	0.140	--	--
Sterigmatocystin (n=8)	--	--	--	--	--	--	-0.484	--	--	--	--
Trichodermol (n=4)	--	--	--	--	--	--	--	--	--	--	--
Verrucarol (n=5)	--	--	--	--	--	--	--	--	--	-0.535	--
GNB											
Endotoxin-LPS (n=16)	**0.623**	--	--	--	--	--	--	--	0.802	--	--
C:10	--	--	--	--	--	--	--	--	--	--	--
C:12	**0.609**	--	--	--	--	--	--	--	**0.732**	--	--
C:14	**0.652**	--	--	--	--	--	--	--	**0.773**	--	--
C:16	**0.681**	--	**-0.459**	--	--	--	--	--	**0.833**	--	--
C:18	**0.580**	--	--	--	--	--	--	--	**0.755**	--	--
Endotoxin-LAL (n=16)	--	--	--	-0.490	0.435	--	0.440	--	0.427	--	--
GPB											
Muramic Acid (n=13)	**0.738**	--	-0.523	--	--	--	--	--	**0.756**	--	--

[1]Abbreviations: LAL- *Limulus* amebocyte lysate assay; LPS- lipopolysaccharide; RH-relative humidity; RS- remediation status; Style-single level or split; Temp-temperature (°C)

[2]Significance: (0.10 > **p < 0.05**) (bold); '--'= p-value not significant

Title:

Fungal Allergens: An Investigation of biomarkers of mold exposure in New Orleans, Louisiana

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Abstract

Background: Post-Hurricane Katrina, New Orleans residents and remediation workers remain exposed to elevated indoor mold spore levels upon entering and gutting water-damaged homes.

Objectives: Our study sought to determine if total and specific Immunoglobulin E (IgE) antibody levels positively correlated with indoor mold exposure duration and severity among post-Hurricane Katrina New Orleans homeowners, volunteers and laborers.

Methods: Questionnaires regarded respiratory health, mask usage and gutting activities. Blood serums were measured for total and mold-specific IgE antibodies. Thirteen dust and thirty-nine air samples were collected using vacuum and Air-O-Cell cassettes, respectively, from 4 undamaged, 6 renovated and 2 non-renovated homes. Pre-and post-gutting blood and environmental samples were analyzed for 5 laborers and 1 previously non-renovated home.

Results: 65% of all participants wore an N95 mask upon initial home inspection post-storm. Eight blood serums had elevated total IgE levels, which were from 2 control and 2 renovated homeowners with low indoor/outdoor mold spore counts, one laborer pre- and post-home gutting and both study dropouts. One homeowner had positive specific IgE responses to 8 molds. *Cladosporium* and *Penicillium/Aspergillus* were the primary genera found in all homes, along with trace amounts of water-indicators *Chaetomium* and *Stachybotrys*. *Aspergillus versicolor* and *Stachybotrys chartarum* were not detected in dust. Chi square exact tests and Spearman exact correlations were not statistically significant for mold exposure, respiratory health, mask usage or Indoor/Outdoor airborne mold spores, based on total IgE levels for all participants.

Conclusions: Neither total nor specific IgE levels correlated with indoor airborne mold exposure severity and duration. Overall, homeowners accounted for most of the elevated total IgE blood serum levels. The study did elucidate some species-specific sensitization in an asthmatic homeowner with doctor-diagnosed mold allergies.

Key Words: Hurricane Katrina, IgE, mold, New Orleans, remediation, respiratory health

Highlights of the study:

- One laborer had elevated total IgE levels before and after gutting a moldy home..

- One renovated homeowner had positive specific IgE responses to 8 molds.

- *Cladosporium* and *Penicillium/Aspergillus* were the primary genera found in homes.

- *Aspergillus* versicolor and *Stachybotrys* chartarum were not found in dust samples.

- Specific IgE levels did not correlate with high indoor airborne mold exposures.

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1.1 Funding Source:

2.1 Introduction[2]

Although there are no standards regulating inhalable mold exposure, the development and exacerbation of asthma and respiratory allergies are associated with dampness, indoor fungal growth and other biological agents (Gaffin and Phipatanakul 2009; Mendell et al 2011). This finding is especially applicable to the estimated 20-40% of the United States population who are genetically predisposed, or atopic, to expressing Type I hypersensitivity to respiratory allergens and others who are sensitized to mold spores after repeated exposures (Horner et al 1995; Rabito et al 2010). Immunoglobulin Type E (IgE) antibodies, which are produced by plasma cells in the skin, lungs and mucosal membranes, are often activated in response to fungal allergens, dander, pollen and other antigens (Janeway et al 2001). Unlike the other 4 antibody isotopes, G, A, M and D, IgE is strongly bound to tissue mast cells, basophils and activated eosinophils. In a person allergic to mold, a cross-reaction of mold-specific antigens and IgE leads to an allergic response within the affected individual. Two early 1970's mold studies conducted in New Orleans found that background levels of ambient air mold spores tended to spike and remain elevated from March until late November, and that the higher mold spore counts coincided with increased reports of hospitalization for asthma in atopic individuals (Salvaggio et al 1971).

Hurricanes Katrina and Rita made landfall in Louisiana on August 29th and September 24th, 2005, respectively; however, even years later, New Orleans residents may be

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[2] Abbreviations: Alt a 1- *Alternaria alternata* allergen; CH-Control home or homeowner; der p 1-Dust mite allergen; IgE-Immunoglobulin E antibodies; I/O-Indoor/Outdoor air mold spore count; kU/L-kilounits per liter (total IgE); kU_A/L-kilounits of antigen per liter (allergen-specific); L-laborer; NCH-Non-control home or homeowner; NRH-Non-renovated home or homeowner; PR-Partially renovated home or homeowner; V-Volunteer

exposed to elevated fungal markers indoors. Relatively year-round high temperatures and humidity in New Orleans encourage mold growth. Residents have been returning to their homes to remove moldy personal items and to remediate their homes through gutting and/or the use of fungicides. The number of homes remediated by the homeowners themselves or with the aid of other family members, compared to those who have hired contractors or volunteers to clean up, is currently unknown. However, studies have shown that of the New Orleans residents and remediation workers interviewed, less than half of them consistently and properly wore N95 respirators during cleanup (Cummings et al 2006).

Our study sought to determine if antibody levels for total and mold-specific IgE would positively correlate with mold exposure duration and severity of indoor mold spore counts among post-Hurricane Katrina New Orleans homeowners, volunteers and laborers. It was assumed that elevated levels of indoor airborne mold spores would elicit measurable mold antibody responses in the non-control and control participants' bloodstreams, and the second assumption was that the molds present four years after Hurricane Katrina were primarily dead, which are still allergenic. To the authors' knowledge, this is the first post-Hurricane Katrina New Orleans study which examines potential mold exposure for remediation workers and homeowners using questionnaires, IgE levels in blood serum samples and environmental air and dust samples. It is also the first study since the 1970's to analyze blood sera of New Orleans residents for multiple mold biomarkers after inhalation exposure (Salvaggio et al 1973).

3.1 Materials and Methods

3.1.1 Participant Selection

This study was approved by Tulane University's Internal Review Board (IRB) prior to recruitment, and all protocols and materials were deemed appropriate for research involving human subjects. For inclusion in the study, participants were required to meet the following criteria: 1) Must be a laborer, volunteer or homeowner currently residing in Orleans or Jefferson Parish, and 2) Must consent to having a 5mL blood sample drawn for mold antibody testing. Volunteers under the age of 18 were permitted to participate as long as written parental consent was given; however, all volunteers who elected to participate were at least 18 years old.

For the control and non-control homeowners, consent was also given to allow air and dust sample collection in the residence. A control home was defined as a residence that did not have any moisture intrusion, via wind- and/or flood-damage, from Hurricane Katrina. The non-control homes were divided into three categories, according to their renovation levels: 1) completely renovated, with no visible signs of mold and/or water damage and replacement of sheetrock and flooring, 2) partially renovated, with removal of damaged sheetrock and flooring, but without the replacement of these building materials, and 3) non-renovated, which still exhibited visible mold and/or water-damage without the replacement of any sheetrock or flooring.

There were 12 homes included in the study: 4 controls, 6 completely renovated, 1 partially renovated and 2 non-renovated. The partially renovated home was initially non-renovated and is included in both categories. Pre-and post-gutting air, dust and blood samples for the laborers were collected. There were 21 participants in the study,

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consisting of 6 laborers, 1 volunteer, 5 control homeowners and 9 non-control homeowners. However, a blood sample was not obtained from the homeowner of the second non-renovated/partially renovated house, due to scheduling conflicts. The homeowner completed a questionnaire and consented to pre-and post-gutting environmental sampling. After the blood samples were obtained, there were 2 dropouts, leaving a total of 19 participants prior to the environmental sampling. One control homeowner and one of the two non-renovated homeowners, who resided in a separate unit on the same premises, voluntarily dropped out.

3.1.2 Questionnaires

Consent forms and a modified version of two standardized questionnaires, the Centers for Disease Control's post-Hurricane Katrina survey and the European Community Respiratory Health Survey, were administered to all participants by trained technicians prior to the blood sample collection (CDC 2006; Janson et al 2001). Control and non-control homeowners answered 13 questions with subparts regarding: the dates of home re-entry to collect personal items or inspect damage, to start cleaning up and to re-occupy the residence; use and type of respiratory protection during re-entry and cleanup; amount of cleanup conducted by homeowner and by a contractor; genetic sensitivity to mold (atopic allergy); prior skin testing; and any doctor-diagnosed or undiagnosed respiratory allergies or asthma in the homeowner or other residents. Laborers and volunteers were asked similar questions about: gutting dates and number of homes gutted; visible mold; the use and type of respiratory protection during cleanup; the same respiratory health questions; and consent to allow follow-up contact concerning future health status.

3.1.3 Blood Sample Collection

A trained phlebotomist collected all blood samples according to the Head-off Environmental Asthma in Louisiana (HEAL) blood-drawing protocol (personal communication). Blood sample collection was conducted in a non-medical office room on Tulane University's campus from late April 2009 to early June 2009. A 23-gauge Safety-Lok needle with luer adapter was used to draw one blood sample per participant into a 5-mL gold –topped Hemogard^{TM} closure tube containing clot activator and serum separator gel (BD Vacutainer® Venous Blood Collection, Franklin Lakes, NJ). Immediately after each sample was collected, the tube was gently inverted five times and allowed to remain upright at room temperature for 30 min. to activate the serum separation gel. In the university laboratory, the samples were centrifuged at 1300 x g for 10 min. to ensure complete separation of the serum from the whole blood and fibrinogen. Three 1-mL aliquots of each serum were drawn, using sterile pipette tips, into 2-mL cryovials. All serum samples were frozen at -20°C and shipped with dry ice to a nationally accredited third-party laboratory for analysis (NIOSH-Allergy and Immunology Division, Morgantown, WV). Participants were compensated with Wal-Mart® gift cards.

3.1.4 Environmental Sampling

Each participant was contacted within 2 to 4 weeks after the blood sample collection to schedule an in-home visit for air and dust sampling. However, with the exception of the laborers, time constraints and conflicts in the participants' schedules resulted in actual home visits being conducted 4 to 23 weeks after the blood sample collections. Sampling

times ranged from early June to late October 2009. For the laborers who partially

renovated one home, air and dust samples were obtained within 4 hours of collecting

blood samples pre- and post-gutting.

Upon entering each home, a walkthrough and visual inspection was conducted to

determine the general environmental conditions. A modified version of the HEAL study's

environmental sampling protocol was followed (personal communication). Either the

participant's bedroom or the living room was selected as the sampling site, according to

the preference of the homeowner or evidence of mold and water damage. The

temperature and relative humidity in the room and 1 meter from the home's front door

were measured with a thermohygrometer pen (Thermo Fisher Scientific, Franklin, MA).

A Surveymaster® protimeter (GE Company, Longmont, CO) was used to measure the

moisture levels in the sample room's walls and floor. Two Air-O-Cell cassettes and

pumps (Zefon International, Ocala, FL) were placed side-by-side on tripods

approximately in the center of the sampling room at a height of 1m and operated

simultaneously for 10 minutes at 15 L/min. The temperature and humidity were also

sampled outdoors, and one Air-O-Cell and pump was setup on a tripod approximately 1

m outside of the front door and operated according to the same protocol as indoor

sampling. A Mighty-Mite canister vacuum cleaner (Eureka, Bloomington, IL) with

single-use allergen filters (EMLab P&K, Phoenix, AZ) entrained in the nozzle was used

to collect two dust samples per home over a five-minute period, according to the HUD

Dust Sample Collection Protocol (HUD 2004). Two additional bulk dust samples, one

from the kitchen and the other from the living room, were collected from the dusty floors

of the partially renovated home, using a small dustpan and broom instead of the vacuum

(Bloom et al 2009). These samples were placed in pre-labeled plastic bags. To avoid

cross-contamination of samples, separate collection instruments were used in each room

of the home. Air samples were placed in study ID-labeled plastic bags and stored at room

temperature until shipped to an accredited third-party laboratory (EMLab P&K, Phoenix,

AZ). Dust samples were stored at -20°C in study ID-labeled plastic bags before they were

shipped to an accredited third-party laboratory (INDOOR Biotechnologies,

Charlottesville, VA).

3.1.5 Laboratory Analyses

Blood serum

The serum samples were analyzed at NIOSH using ImmunoCAP® Allergy testing

(Pharmacia Diagnostics AB-Phadia, Uppsala, Sweden) for the presence of total IgE

antibodies to 4 mold genera: *Alternaria, Aspergillus, Cladosporium* and *Penicillium.*

Screening for specific IgE antibodies was conducted for the following 10 mold species

commonly found in the southeast region: *Alternaria alternata, Aspergillus fumigates,*

Aspergillus niger, Chaetomium globosum, Cladosporium herbarium, Curvularia lunata,

Epicoccum purpurascens, Fusarium proliferatum, Penicillium notatum, Trichoderma

viride (Phadia 2011). ImmunoCAP® processes used to analyze the serum samples for

antibodies to the 10 mold allergens are detailed elsewhere (Lam 2008).

Air

EMLab P&K characterized the non-viable or dead mold spores present in each Air-O-

Cell spore trap by genera or group and quantified the levels and percentages of mold

spores and fungal hyphal fragments per cubic meter of air sampled through microscopic

examination. An overview of the non-biological background debris evident was also noted in the lab analysis, and a determination of 4+ indicated the sample count given was to be interpreted as a minimum count.

Dust

INDOOR Biotechnologies applied allergen-specific Enzyme-linked Immunosorbent Assays (ELISAs) and Multiplex Array for Indoor Allergens (MARIA™) in the dust analysis (Earle et al 2007). Monoclonal antibodies covalently coupled to fluorospheres were used to test for the presence of *Aspergillus versicolor* and *Stachybotrys chartarum* in the house dust. Per two participants' requests, the dust samples for 1 control homeowner and 1 renovated homeowner were also analyzed for Der p 1 and Alt a 1. The two bulk dust samples from the partially renovated home were also included in the dust analysis.

3.1.6 Statistical Analyses

All data related to questionnaire responses from 21 participants, blood sample data from 20 participants and 39 air samples from 13 homes (26 indoor and 13 outdoor samples collected-one home in different stages of repair was counted twice) study were summarized using descriptive statistics. Mean I/O spores were calculated using Excel® (Microsoft Excel® 2007, Redmond, WA). SPSS was used to determine all remaining analyses, such as descriptive statistics (means, medians and interquartile ranges) for the control and non-control homeowners' total IgE levels and I/O spores, and pre-and post-gutting total IgE levels in 5 laborers (IBM-SPSS vers. 19.0, Somers, NY). A modified variable coding system from a similar study was implemented to adequately compare

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mold exposure, respiratory health, mask usage, total IgE and I/O spores for each home (Cummings et al 2008). Mold exposures were categorized in the following manner: 0 = no water intrusion in home and no cleanup activities; 1 = no water intrusion in home, but participated in cleanup activities; 2 = water intrusion in home, but did not participate in cleanup activities; 3 = water intrusion in home and participated in cleanup activities; and 4 = participated in cleanup of more than 1 home (laborers and volunteers only). Mask usage during cleanup/gutting was indicated by: 0 = not applicable; 1 = Yes; and 2 = No. Respiratory health scores were assigned scores of: 0 = no respiratory problems reported; 1 = undiagnosed respiratory allergy; and 2 = doctor-diagnosed respiratory allergy. Indoor/outdoor air mold spore ratios were included as: 1 = less than 1 (indoor count below outdoor count) and 2 = 1 or greater (indoor count is equal to or exceeds the outdoor count). Chi square exact tests and Spearman rho exact correlations (as a nonparametric method for non-normally distributed variables) were used to compare all 4 categories with total IgE levels, which were categorized according to: 0 = not applicable; 1 = normal; and 2 = elevated. Total IgE data collected for the 5 laborers at two time points were categorized as either 1 = normal or 2 = elevated. Wilcoxon signed rank test was used to determine any median differences between the pre-and post-gutting serum total IgE levels. All study hypotheses were tested throughout the analysis at the 5% level of significance. Estimates of percentage and their 95% confidence intervals were determined.

4.1 Results

4.1.1 Self-reported mold exposures and health

Neither *Aspergillus versicolor* nor *Stachybotrys chartarum* was detected in the dust samples. Alt a 1 and der p 1, *Alternaria* and dust mite allergens respectively, were also absent from the two dust samples analyzed. NRH1 and CH2 dust samples were positive for *S. chartarum*; however, the amounts were negligible and were well below the limit typically reported by the lab (Smith, B, personal communication).

Questionnaire data, which identify some potential sources of indoor air mold exposure for homeowners, laborers and volunteers, are summarized in Table 1. The table is a modified version of one from another study (Sordillo et al 2011). Questionnaire and environmental sampling data for the non-blood-sampled homeowner, NRH2/PR, were included in the tables. Only 3 renovated and non-renovated homeowners, RH1, RH2 and NRH1, reported participating in gutting or removing moldy belongings from any homes post-storm. However, there was one case of overlap between the non-control homeowner and laborer categories. RH1's participant assisted in completely gutting and renovating the home, which received an estimated 3 feet of floodwater. The participant also co-owned a family-operated construction company and was hired as a laborer, L5, to gut NRH2/PRH approximately 5 weeks after the initial blood draw. 65% of all respondents wore an N95 mask upon first entering the home to inspect for damage and/or collect non-moldy belongings.

71% of the homeowners who performed any amount of storm-related cleanup or repairs on their property reported wearing an N95 mask, and 91% of all respondents who participated in gutting activities on their home or other properties used an N95 mask.

Only one participant, RH3, reported having doctor-diagnosed mold allergy and asthma. Questionnaire and blood serum data for the two dropouts were included in the study tables. Of the two dropouts, the second homeowner of the first non-renovated house reported having an unconfirmed non-mold-specific respiratory allergy, which had not caused any problems in the past two years. Neither of the dropout homeowners was present during any cleanup activities, and neither participated in gutting activities in any homes.

Total and mold-specific IgE data are reported in Table 2. 40% of the 20 blood-draw participants exhibited elevated levels of total IgE. Both dropouts had elevated total IgE serum levels, but their indoor environmental exposures for comparison were not measured. Only one participant, RH3, had both elevated total and specific IgE levels, the latter of which is shown in Table 3. Positive specific IgE responses were present for 8 mold species, excluding only *Aspergillus niger* and *Chaetomium globosum*. The strongest antibody generation was to *Alternaria alternata*. The participant also noted having doctor-diagnosed allergy-induced asthma. No hospitalizations or non-routine doctor's visits for asthma were noted, but an albuterol inhaler was used once in June 2009 due to sneezing and wheezing.

Mean I/O spores are summarized in Table 4. The only non-control home with mean I/O spores less than 1 was RH3. Although the second smallest non-control I/O spores belonged to a non-renovated home, NRH1, the indoor mold spore count per cubic meter was still more than 6 times greater than the highest indoor mold spore count for the renovated homes, RH2. Figure 1 shows the composition of these indoor and outdoor airborne mold spore counts in terms of fungal genera. Ascospore and basidiospore groups

were found inside of 77% and outside of 100% of the control and non-control homes. However, the airborne mold genera of interest in this study were those which comprised the 10-mold blood serum panel and dust sample analyses. *Cladosporium* and *Penicillium/Aspergillus* spore counts per cubic meter were present in the largest concentrations; all other genera were found in trace amounts.

Descriptive statistics, specifically means, medians and interquartile ranges, for the groups' total IgE levels and I/O spores are shown in Table 5. With the exception of one laborer, one renovated homeowner and the volunteer, the control homeowner group had the highest median total IgE level. The sole control homeowner who reported having a respiratory allergy, CH2, had a suspected allergy to pollen, which frequently flared up after moving into the residence approximately 3 months prior to the blood draw. The homeowner had the lowest level of total IgE, 4.57 kU/L, in the study, and the highest I/O, 0.29, for the controls. Chi square exact tests and Spearman rho exact correlations, respectively, were not statistically significant for mold exposure ($p = 0.277$; $p = 0.480$), respiratory health ($p = 0.314$; $p = 0.908$), mask usage ($p = 0.434$; $p = 0.623$) or I/O spores ($p = 0.222$; $p = 0.160$), based on total IgE levels for all participants.

4.1.2 Pre-and post-gutting serum IgE levels for laborers of NRH2/PR

The median total IgE levels for the pre-and post-gut laborers were well within the normal range. Only one laborer had elevated total IgE levels pre-and post-gutting, although this was not the laborer who reported having an undiagnosed respiratory allergy. For the Wilcoxon signed rank test, there was no statistically significant difference between the distribution of pre-and post-gutting total IgE serum levels for the laborers.

5.1 Discussion

5.1.1 Post-Hurricane Katrina Studies and Mold Exposure

Post-Hurricane Katrina New Orleans studies published to date have examined viable and non-viable fungal by-products in air and dust, bacterial and dust mite indicators and mold allergy via skin prick testing (Adhikari et al 2009; Adhikari et al 2010; Chew et al 2006; Lee et al 2006; Rabito et al 2010; Rao et al 2007; Riggs et al 2008; Solomon et al 2006). However, to the authors' knowledge, there have been no Hurricane Katrina-related studies published which examine the blood sera, air and dust samples of interviewed remediation workers and residents in New Orleans, LA. In light of increased natural disasters and recent household flooding nationwide, this study provides pertinent information for assessing potential inhalation mold exposures and mold-specific antibody responses in exposed residents and remediation workers.

The greatest concern regarding mold exposure and mask usage after Hurricane Katrina was for those entering water-damaged homes to inspect and collect salvageable belongings, residents who renovated their home themselves and for the laborers and volunteers who were gutting from 3 to 20 or more homes. As stated in early post-storm studies, at least 44-46% of New Orleans' homes had extensive mold growth, which would potentially impact the health of residents and workers exposed to the environmental conditions (CDC 2006; Riggs et al 2008). Fungal fragments, mold spores, mycotoxins, bacterial components and other biological contaminants may become aerosolized and inhaled when dust and building materials are disturbed (Adhikari et al 2009; Bloom et al 2009). It was unknown whether residents knew how to properly don N95 respirators while cleaning or gutting, as was the case for only 24% of a group of

New Orleans residents (Cummings et al 2007). More than half of those in our study wore an N95 mask upon first entering their home post-Katrina and during cleanup. This was consistent with 63% of residents surveyed in a larger post-Katrina study who wore N95 masks while gutting homes (Cummings et al 2008).

Reported respiratory allergies and exacerbation due to mold exposure were not prevalent in this study. Only 33% of the participants had either a diagnosed or an undiagnosed respiratory allergy, such as hay fever, and the only asthmatic participant, RH3, had doctor-diagnosed allergy-induced asthma. The participant also had the sole positive mold-specific IgE test, which supported the clinical diagnosis. Otherwise, a single positive specific IgE test would have been insufficient to indicate that a participant has a respiratory allergy or is atopic to mold (Bardana 2003; Bush 2008; Gergen et al 2009). All respiratory allergies were present before the storm, and only three participants with respiratory allergies reported experiencing exacerbation of symptoms, e.g. sneezing and wheezing, post-Katrina. All participants were asked if they were atopic, genetically predisposed, to mold and other respiratory allergens based on their parents' health status. Only one participant, RH5, had a deceased father with doctor-diagnosed asthma; however, the homeowner did not report experiencing any respiratory distress pre- or post-storm. Most of those with respiratory allergies resided in homes that flooded during Hurricane Katrina and had been renovated at the time of sampling.

Four years or more after the storm, it was expected that mold spore levels would not be reminiscent of the thousands of spores and elevated I/O spores found in non-renovated, water-damaged homes three months after the disaster (Chew et al 2006; Solomon et al 2006). However, with the exception of RH3, all of the renovated, partially and non-

renovated homes still had indoor airborne mold spore levels that exceeded outdoor spore counts. The control homes exhibited much lower indoor mold spore counts than outdoors, which was consistent with other homes in southeastern United States without moisture intrusion or visible mold growth (Horner et al 2004; Lee et al 2006). As with previous studies, the primary fungal genera found in homes were soil fungi *Cladosporium* and *Penicillium/Aspergillus* (Chew et al 2006; Lee et al 2006; Rao et al 2007; Riggs et al 2008; Solomon et al 2006). Trace amounts of water-indicator mold genera, *Stachybotrys* and *Chaetomium*, were found in and/or around RH2 and pre-and post-gutting of NRH2/PR. Both homes had received at least 4 feet of flood water from the storm. Although RH2 had been renovated and was occupied during sampling, the homeowners owned a construction company and had gutted over 20 additional homes. Some of the fungi from other homes may have been carried into their home on clothing or equipment. The predominant mold group found in the homes was basidiospores, which have been present throughout New Orleans' environment and may contribute to respiratory concerns (Salvaggio et al 1973). For the positive specific IgE result, RH3 had a Class III (high) reaction to *Alternaria alternata*, which was not present in either the indoor or the outdoor air sample of the participant's residence. The corresponding dust sample was also negative for Alt a 1, although sampling was conducted exactly six weeks after the blood draw. It is assumed that the participant's Class II (medium) responses to *Aspergillus*, *Cladosporium* and *Penicillium* species, also the most prevalent genera in and around the house during the fall sampling date, contributed to the exacerbation of asthma and allergies experienced pre- and post-storm.

5.1.2 Research Limitations

This study has limitations which may partially account for the lack of any statistically significant result. The small sample size, 21 blood samples and 13 environmental samples, may have hindered the ability to observe correlations between respiratory health effects and environmental factors. Also, the 10-minute air sampling time may not have been long enough to thoroughly characterize the microbial contaminants in the environment. However, other studies also did not find any significant associations between post-storm respiratory symptoms and elevated residential mold exposure (Barbeau et al 2009; Bardana 2003; Rabito et al 2010; Wiszniewska et al 2009). Serum IgE to other common household allergens, such as dust mites, cockroaches, rodent droppings and pet dander, were not analyzed as with more comprehensive studies; the only exceptions were the two homes analyzed for 1 dust mite allergen (Salo et al 2011). There was some difficulty finding homeowners, contractors and volunteer organizations a few years after the storm willing to allow blood and environmental sample collection, especially for homeowners who had not yet gutted their water-damaged homes at the study's onset. Water-damaged homeowners with funding tended to have gutted their homes within the first 2-3 years post-storm, although an exact figure for total homes gutted is unknown. Some of the homes which were still ungutted were sold to Louisiana's The Road Home® program, were demolished or remained in various stages of disrepair with displaced homeowners.

Elevated total IgE levels may have been caused by an allergic reaction to mold species not included in the 10-panel selection, and it is difficult to pinpoint exactly where and when mold exposures occur. For laborers, the use of an N95 respirator may help explain

the relatively low IgE levels observed, and there was one case of overlap between laborers and homeowners.

6.1 Conclusions

Total IgE levels did not correlate with residential airborne mold exposure severity and duration.. One positive specific IgE mold test confirmed the clinical diagnosis of one participant's mold allergy-induced asthma; in-home environmental sampling may have accounted for most mold-specific IgE reactions observed. Pre-and post-gutting serum IgE levels for 5 laborers did not indicate significant mold exposure and remained relatively stable over the one-week lapse between blood sample collections. According to the questionnaire data, the rate of mask usage while gutting was very high (91%) and respiratory allergies were low (33%) post-Katrina. Overall, no association was found between post-Katrina indoor air mold exposures and elevated IgE serum levels. In light of recent natural disasters and flooding worldwide, this study provides information for assessing potential inhalation mold exposures and antibody responses in exposed communities. This study lays the foundation for future studies incorporating questionnaires, serum IgE and environmental sampling, preferably within months of these disasters.

7.1 Acknowledgements

8.1 References

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9.1 Tables   Table A.1. Summary of questionnaire responses

Subject	Characteristic	Controls (n=5)	Non-control Homeowners (n=9)			Laborers (n=6)	Volunteer (n=1)
			Renovated (n=6)	Partially renovated (n=1)	Non-renovated (n=2)		
Home Re-entry	1st entered home & cleanup dates	9/05 - 10/05	9/05 – 6/06	10/05-6/09	9/05-10/05	n/a	n/a
	1st Occupied Home (overnight stay)	9/05 - 10/05	6/06 - 5/09	None	None	n/a	n/a
Exposure	Estimated Flood level	0	0 - 9 ft.	7 ft.	0	n/a	n/a
	Contractor cleanup	n/a	3	1	1	n/a	n/a
	Present during cleanup	n/a	1	0	1	n/a	n/a
	Homeowner cleanup (%)	n/a	95	0	100	n/a	n/a
	Gutted any homes	None	10/05- 6/09	None	2006	10/05- 6/09	12/05- 7/07
	Number of homes gutted	0	1 and 20+	0	2	1-20+	5
	Visible mold-cleanup	n/a	Yes-20+	n/a	Yes	Yes-20+	Yes-5
Mask Usage	1st entered home	0	3	1	2	6	1
	During cleanup	n/a	4	n/a	1	n/a	n/a
	During gutting	n/a	2	n/a	1	6	1
Health	Respiratory allergies (dd)	1(No)	3(Yes)	1(No)	1(No)	1(No)	No
	Mold allergy (dd)	No	1(Yes)	No	No	No	No
	Asthma (dd)	No	1(Yes)	No	No	No	No
	Allergy symptoms in home	Yes-frequently	2	No	No	No	n/a

[a]. Abbreviations: dd-doctor-diagnosed; parentheses indicate undiagnosed or doctor-diagnosed condition.

Table A.2. Blood serum total and mold-specific IgE antibody levels

| Antibody Test | Measurement | Controls (n=5) | Non-control homeowners (n=8) | | Laborers (n=6) | | | |
			Renovated (n=6)	Non-renovated (n=2)	Pre-gut Laborers (n=5)	Post-gut Laborers (n=5)	Laborer (n=1)	Volunteer (n=1)
Total IgE[b]	Normal ($\leq$ 100)	2	4	1	4	4	1	0
Reference Levels (kU/L)	Elevated (>100)	3	2	1	1	1[d]	0	1
Specific IgE	Class 0 (< 0.35)	5	5	2	5	5	1	1
Evaluation Class (kU$_A$/L)	Class I -VI (0.35 –100)	0	1	0	0	0	0	0

[a.] Abbreviations: IgE-Immunoglobulin Type E antibodies; kU/L-kilounits per liter; kU$_A$/L-kilounits of antigen per liter (allergen-specific)

b. Serums were exposed to *Alternaria, Aspergillus, Cladosporium* and *Penicillium* to measure total IgE.

[c.] ImmunoCAP® analysis groups specific IgE results into 6 classes: Class 0 (<0.35); Class I (0.35-0.70)-Low; Class II (0.7-3.5)-Medium; Class III (3.5-17.5)-High; Class IV (17.5-50)-Very High; Class V (50-99)-Very High; Class VI ($\geq$ 100)-Very High.

[d.] The same laborer had elevated pre- and post-gutting total IgE levels.

Table A.3. Positive Blood Serum Specific IgE mold antibody test data

sIgE	A. alternata	A. fumigatus	A. niger	C. globosum	C. herbarium	C. lunata	E. purpurascens	F. proliferatum	P. notatum	T. viride
kU_A/L	13.7	0.94	<0.35	<0.35	0.87	0.87	2.64	1.74	0.87	0.97
Eval. Class	III	II	0	0	II	II	II	II	II	II

[a]. Abbreviations: Eval. Class: Evaluation Class; sIgE: specific Immunoglobulin Type E antibodies

[b.] Fungal genera (in the following order) are: *Alternaria* alternata, *Aspergillus* fumigatus and niger, *Chaetomium* globosum, *Cladosporium* herbarium, *Curvularia* lunata, *Epicoccum* purpurascens, *Fusarium* proliferatum, *Penicillium* notatum and *Trichoderma* viride; kUA/L – kilounits of antigen per liter (allergen-specific)

[c.] ImmunoCAP® analysis groups specific IgE results into 6 classes: Class 0 (<0.35); Class I (0.35-0.70)-Low; Class II (0.7-3.5)-Medium; Class III (3.5-17.5)-High; Class IV (17.5-50)-Very High; Class V (50-99)-Very High; Class VI (≥ 100)-Very High.

Table A.4. Indoor and outdoor airborne mold spore counts grouped by home status

| Home Status | Mold Spore Counts (spores/m^3) | | |
	Indoor	Outdoor	Mean I/O
Control Homes (n=4)			
CH1	28	410	0.07
CH2	60	210	0.29
CH3	8	1500	0.00
CH4	54	980	0.06
Renovated Homes(n=6)			
RH1	517	310	1.67
RH2[b]	5600	1100	5.09
RH3	54	160	0.34
RH4	1660	1500	1.11
RH5	2216	1100	2.01
RH6[b]	1641	850	1.93
Non-renovated Homes (n=2)			
NRH1[b]	36036	34000	1.06
NRH2 (pre-gut)[b]	65722	310	212.01
Partially renovated Home (n=1)			
PRH (post-gut)[b]	4000	440	9.09

[a.] Abbreviations: I/O- Indoor/Outdoor

[b.] High levels of non-biological background debris were detected in the indoor and/or outdoor samples, and these are regarded as minimal counts.

[c.] The indoor spore counts consist of the total number of spores found in 2 Air-O-Cell samples. Samples less than the detection limit were counted as one spore per cubic meter.

[d.] Mean I/O was calculated by dividing indoor spore counts by outdoor spore counts.
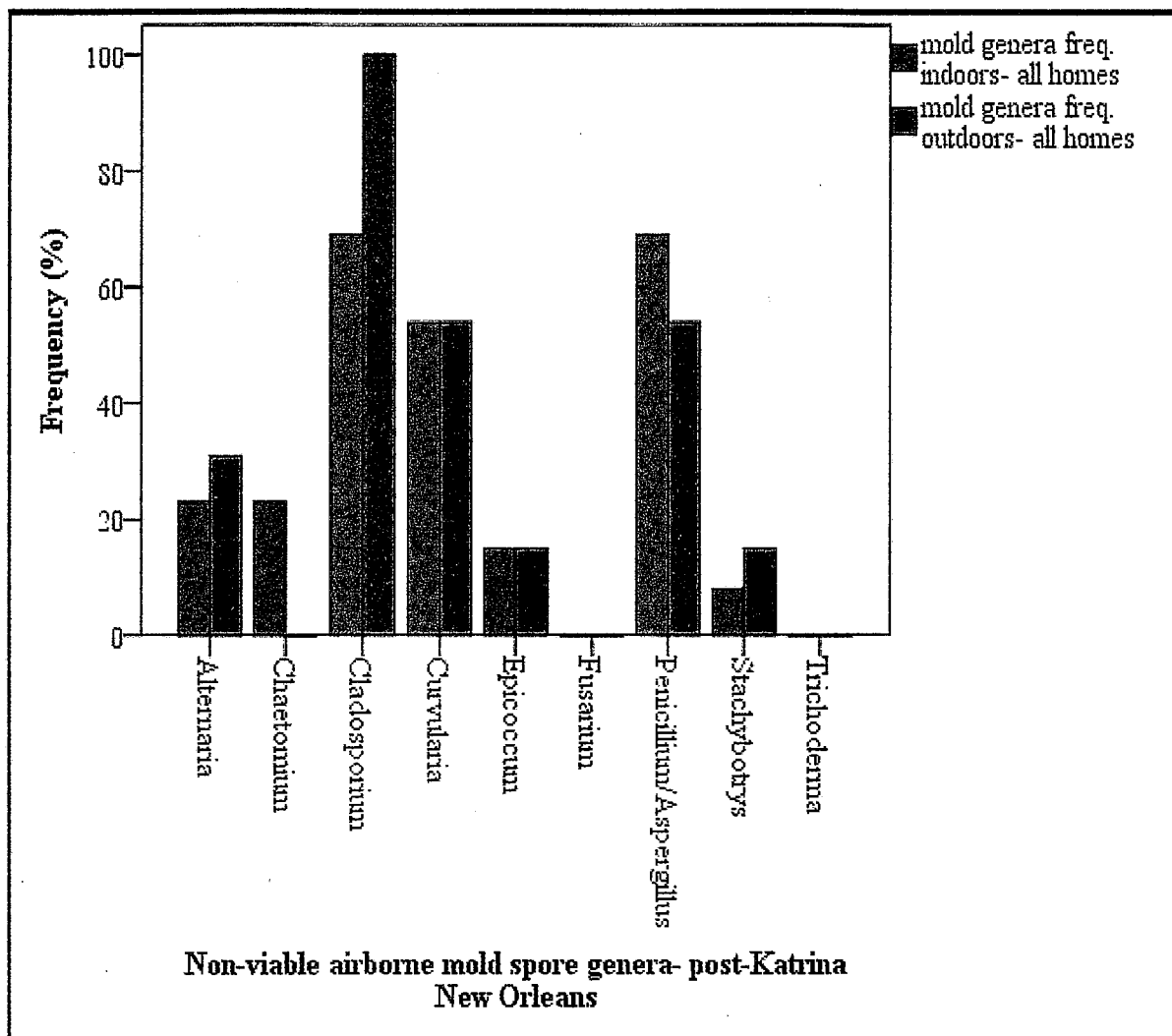
Table A.5. Descriptive statistics for control and non-control homeowners, laborers and volunteer

Status	Number of subjects		Descriptives		
Group	n	Variable	Mean	Median	Interquartile Range
Control Homeowner	5	Total IgE (kU/L)	102.91	130.00	10.79 – 181.50
	4	I/O air	0.11	0.07	0.02 – 0.24
Renovated Homeowner	6	Total IgE (kU/L)	449.37	53.35	30.38 – 728.00
	6	I/O air	2.03	1.80	0.92 – 2.78
Non-renovated Homeowner/Partially renovated homeowner	2	Total IgE (kU/L)	100.85	100.85	86.70 – 100.85
	2	I/O air	74.05	9.09	1.06 – 9.09
Laborers and Volunteers	2	Total IgE (kU/L)	147.75	147.75	31.50 – 147.75
Laborers (pre-gut)	5		83.38	55.50	30.5 – 150.15
Laborers (post-gut)	5		84.80	55.60	30.5 - 153.75

[a] Abbreviations: IgE-Immunoglobulin Type E antibody; I/O-indoor/outdoor air mold spore ratio; kU/L-kilounit per liter

Figures

Figure 1. Color Figure (journal's web edition only)- Frequencies of mold genera in indoor (A) and outdoor (B) air samples collected in control, renovated, non-renovated and partially renovated homes combined

a. Frequencies: percentages are determined by the number of control and non-control homes which exhibited any amount of the particular mold genera.
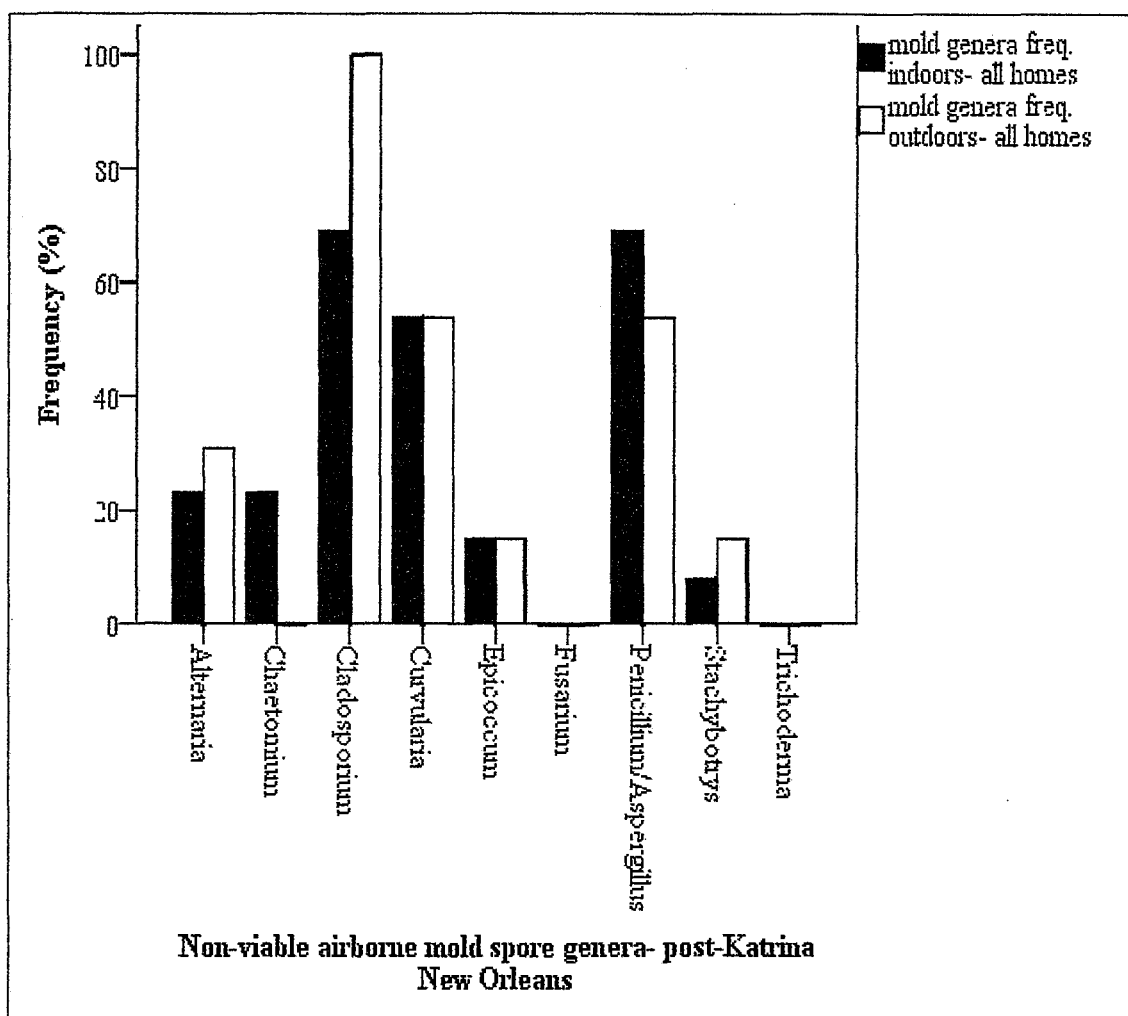
b. The mold genera listed were also included in the 10-mold species laboratory blood panel, with the exception of *Stachybotrys*; the dust samples were analyzed for *Aspergillus* and *Stachybotrys*.

c. *Penicillium* and *Aspergillus* are difficult to distinguish using fungal spore counts, so they are classified as one group for non-viable sampling analysis.

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Figure Captions

Figure 1. Black and White Figure (for printed journal edition) - Frequencies of mold

genera in indoor (A) and outdoor (B) air samples collected in control, renovated, non-

renovated and partially renovated homes combined

Legend:
- mold genera freq. indoors- all homes
- mold genera freq. outdoors- all homes

Y-axis: Frequency (%)

X-axis categories: Alternaria, Chaetomium, Cladosporium, Curvularia, Epicoccum, Fusarium, Penicillium/Aspergillus, Stachybotrys, Trichoderma

Non-viable airborne mold spore genera- post-Katrina New Orleans

[a] Frequencies: percentages are determined by the number of control and non-control homes which exhibited any amount of the particular mold genera.

[b] The mold genera listed were also included in the 10-mold species laboratory blood panel, with the exception of *Stachybotrys*; the dust samples were analyzed for *Aspergillus* and *Stachybotrys*.

[c] *Penicillium* and *Aspergillus* are difficult to distinguish using fungal spore counts, so they are classified as one group for non-viable sampling analysis.

Appendix B. Supplemental Research Information

B1. Questionnaires

Pre-Katrina Mold Assessment Characteristics

Date: Sex: Child or Adult: Site/Location:

1. Has your home ever flooded before?   Yes or No

If yes, has home flooded in past 10 years?   Yes or No

2. Have you had visible mold growth in the past?   Yes or No

3. Do you currently have visible mold growth in the home?   Yes or No

4. Do you have any leaks or moisture in your home?   Yes or No

5. Do you own any furred pets?   Yes or No

6. Do you own any feathered pets?   Yes or No

7. Do you have plants in the home?   Yes or No

8. Do you have air conditioning in the home?   Yes or No

Central or Window

9. Do you use a humidifier?   Yes or No

10. Do you use a Dehumidifier?   Yes or No

11. Doctor-diagnosed asthma?   Yes or No

12. Do you or anyone in your home smoke?   Yes or No

13. Any asthmatic children live in the home?   Yes or no

14. Have you gone to the ER in the past 12 months for breathing problems?   Yes or No

15. Hospital admission in past 12 months for breathing problems?   Yes or No

16. How many wheezing events per year? a. None b. 1-3 c. 4-12 d. >12 e. unknown

17. Does physical activity cause breathing problems, cough or wheeze?   Yes or No

If yes, a. sometimes b. usually or c. always

18. How often is the inhaler or nebulizer used for these problems?

a. Rarely or only with exercise   b. 4 or more times per month c. daily use

d. more than once per day

19. Have you ever had skin testing conducted?   Yes or No

If so, for what? a. dust mite b. trees c. grass d. ragweed e. cat f. dog g. molds

h. cockroach

Questionnaire for all late post-Katrina homeowners

Home Occupancy Status

1. On what day/date did you first re-enter the building following Hurricane Katrina or Rita? _(dd/mm/yy)_____ (see calendar)

2. Did you use respiratory protection when you first entered the building to inspect damage or collect personal belongings? Yes_____ No_____

    2a. If yes, what type? _____

3. How much flood water did the home receive? _____

4. On what day/date did you begin work on cleaning and/or repairing the building? _(dd/mm/yy)_____

5. Did you hire a contractor or company to do any clean up or repairs? Yes ____ No____

    5.a. Were you present during the clean up and repair? Yes_____ No _____

    5.b. Were other members of the household present during cleaning and repair? Yes ____ No _____

    5.b. Did you wear respiratory protection while work was being done? Yes __ No __

        5.b.1 If yes, what type?_____

6. Estimate the percentage of the clean up and repair you did yourself_____%

    6.a. Did you use respiratory protection while you did the clean up and repair? _____Yes _____No

    6.b. If yes, what type? _____

7. Have you conducted home gutting or mold remediation since Hurricane Katrina? Yes_____ No_____

    7a.If yes, how many homes? __(dd/mm/yy)_____

    7b. When was the last time you gutted or did mold remediation? date__(dd/mm/yy)_____

8. Are you currently occupying your home (staying overnight)? Yes___ No___

8.a. If yes, on what day/date following the hurricane did you move into your home? _(dd/mm/yy)_____

8.b. If no, on what day/date do you anticipate staying in your home?
_____

9. Do you plan to occupy this building sometime over the next year? Yes____ No____

Health Status of Homeowner

10. Do you have hay fever or allergies to things that you breathe?   Yes____ No____
(if "yes", continue; if "no" go to question *asthma*)

10.a. Are your allergies doctor diagnosed?   Yes____ No____

10.b. Are you allergic to molds?   Yes____ No____   Don't know____

10.b.1. If yes, has this been diagnosed by a doctor (for example, a skin prick test)? Yes____ No____

11. Do you have asthma?   Yes____ No____   (if "yes", continue; if "no", stop: "Thank You")

11.a. Is your asthma doctor-diagnosed?   Yes____ No____

11.b. Are you allergic to mold or mildew?   Yes____ No____ Don't know____

11.c. When was your last asthma episode that required use of an inhaler? (dd/mm/yy)
_____ ; If you don't recall the date, how long ago?

_____

11.d. When was your last asthma episode that required an emergency visit to a doctor's office or emergency room? (dd/mm/yy)_____; If you don't recall the date, how long ago? _____

12.a. Are you currently experiencing allergy symptoms while in this building?   Yes____ No____

12.b. Are you currently experiencing asthma symptoms while in this building?   Yes____ No____

12.c. Have you experienced allergy symptoms since the hurricanes at any time while in this building?
                 Yes____ No____
If so, how frequently? Frequently _____   Sometimes _____ Rarely _____

12.d. Have you experienced asthma symptoms since the hurricanes at any time while in this building?

Yes___ No___

If so, how frequently? Frequently _____ Sometimes _____ Rarely _____

13. Are there any other heath related symptoms you have been experiencing since the hurricanes?

These could be either existing conditions with increased frequency or severity or newly developed conditions.

Please describe

Health Status of Others in the Home

Person #_____ (enter numbers in sequence, starting with "2")

How is this person related to you? _____ (e.g., oldest son)

10. Does this person (or, for example, "your son") have hay fever or allergies to things he/she breaths? Yes___ No___ (if "yes", continue; if "no" go to question 5)

10.a. Are this person's (or, for example, "your son's") allergies doctor diagnosed? Yes___ No___

10.b. Are this person's (or, for example, "your son's") sensitive to molds? Yes___ No___

10.b.1. If yes, has this been diagnosed by a doctor (for example, with a skin prick test)?
Yes___ No___

11. Does this person (or, for example, "your son") have asthma? Yes___ No___ (if "yes", continue; if "no", stop: "Thank You")

11.a. Is this person's (or, for example, "your son's) asthma doctor-diagnosed? Yes___ No___

11.b. Is this person (or, for example, "your son) allergic to molds or mildew? Yes___ No___ Don't know___

11.c. When was this person's (or, for example, "your son's) last asthma episode that required use of an inhaler? (dd/mm/yy) _____ ; If you don't recall the date, how long ago? _____

11.d. When was this person's (or, for example, "your son's) last asthma episode that required an emergency visit to a doctor's office or emergency room?

(dd/mm/yy)_____; If you don't recall the date, how long ago?
_____

12.a. Is this person currently experiencing allergy symptoms while in this building?
Yes___ No___

12.b. Is this person currently experiencing asthma symptoms while in this building?
Yes___ No___

12.c. Has this person experienced allergy symptoms since the hurricanes at any time while in this building?
             Yes___ No___
             If so, how frequently? Frequently _____ Sometimes _____ Rarely
_____

12.d. Has this person experienced asthma symptoms since the hurricanes at any time while in this building?
             Yes___ No___
             If so, how frequently? Frequently _____ Sometimes _____ Rarely
_____

13. Are there any other heath related symptoms they have been experiencing since the hurricanes?
    These could be either existing conditions with increased frequency or severity or newly developed conditions.
             Please describe

Questionnaire for late post-Katrina volunteers

Volunteer Questionnaire

1. On what day/date did you begin home gutting in the New Orleans area?
   (dd/mm/yy)_____

2. How many homes have you gutted?_____

3. Did you wear respiratory protection during gutting activities? Yes_____ No_____

   3a.If yes, what type _____

4. Did you wear other personal protective gear (e.g., goggles, coveralls, gloves)?
   Yes_____ No_____

5. Did the homes have visible mold present? Yes_____ No_____

   5a. If yes, how many had mold present? _____

6. When was the last time you did home gutting or mold remediation?
   (dd/mm/yy)_____

Health Status of Volunteer

10. Do you have hay fever or allergies to things that you breathe? Yes___ No___
      (if "yes", continue; if "no" go to question *asthma*)

   10.a. Are your allergies doctor diagnosed? Yes___ No___

   10.b. Are you allergic to molds? Yes___ No___   Don't know___

      10.b.1. If yes, has this been diagnosed by a doctor (for example, a skin prick test)?
      Yes___ No___

11. Do you have asthma? Yes___ No___   (if "yes", continue; if "no", stop: "Thank You")

   11.a. Is your asthma doctor-diagnosed? Yes___ No___

   11.b. Are you allergic to mold or mildew? Yes___ No___ Don't know___

11.c. When was your last asthma episode that required use of an inhaler? (dd/mm/yy)
_____ ; If you don't recall the date, how long ago?

_____
11.d. When was your last asthma episode that required an emergency visit to a doctor's office or emergency room? (dd/mm/yy)_____; If you don't recall the date, how long ago? _____

12.a. Are you currently experiencing allergy symptoms while in this building?   Yes____ No____

12.b. Are you currently experiencing asthma symptoms while in this building?   Yes____ No____

12.c. Have you experienced allergy symptoms since the hurricanes at any time while in this building?
       Yes____ No____
       If so, how frequently? Frequently _____   Sometimes _____ Rarely _____

12.d. Have you experienced asthma symptoms since the hurricanes at any time while in this building?
       Yes____ No____
       If so, how frequently? Frequently _____   Sometimes _____ Rarely _____

13. Are there any other heath related symptoms you have been experiencing since the hurricanes?
    These could be either existing conditions with increased frequency or severity or newly developed conditions.
       Please describe

14. (To be asked of home gutting volunteers) Would you mind if we contact you in the future to follow up on your health status ?   Feel free to contact me_____   Do not contact me_____

Name: _____   Phone:

_____

Address:

_____

Email: _____

Questionnaire for late post-Katrina laborers

Laborer Questionnaire

1. On what day/date did you begin home gutting in the New Orleans area?
   (dd/mm/yy)_____

2. How many homes have you gutted?_____

3. Did you wear respiratory protection during gutting activities? Yes ____ No____

   3a.If yes, what type _____

4. Did you wear other personal protective gear (e.g., goggles, coveralls, gloves)?
   Yes_____ No_____

5. Did the homes have visible mold present? Yes ____ No_____

   5a. If yes, how many had mold present? _____

6. When was the last time you did home gutting or mold remediation?
   (dd/mm/yy)_____

Health Status of Laborer

10. Do you have hay fever or allergies to things that you breathe?   Yes___ No___
    (if "yes", continue; if "no" go to question *asthma*)

    10.a. Are your allergies doctor diagnosed?   Yes___ No___

    10.b. Are you allergic to molds?   Yes___ No___   Don't know___

       10.b.1. If yes, has this been diagnosed by a doctor (for example, a skin prick test)?
       Yes___ No___

11. Do you have asthma?   Yes___ No___   (if "yes", continue; if "no", stop: "Thank You")

    11.a. Is your asthma doctor-diagnosed?   Yes___ No___

    11.b. Are you allergic to mold or mildew?   Yes___ No___ Don't know___

11.c. When was your last asthma episode that required use of an inhaler? (dd/mm/yy) _____ ; If you don't recall the date, how long ago?

_____

11.d. When was your last asthma episode that required an emergency visit to a doctor's office or emergency room? (dd/mm/yy)_____; If you don't recall the date, how long ago? _____

12.a. Are you currently experiencing allergy symptoms while in this building?   Yes___ No___

12.b. Are you currently experiencing asthma symptoms while in this building?   Yes___ No___

12.c. Have you experienced allergy symptoms since the hurricanes at any time while in this building?
    Yes___ No___
    If so, how frequently? Frequently _____ Sometimes _____ Rarely _____

12.d. Have you experienced asthma symptoms since the hurricanes at any time while in this building?
    Yes___ No___
    If so, how frequently? Frequently _____ Sometimes _____ Rarely _____

13. Are there any other heath related symptoms you have been experiencing since the hurricanes?
    These could be either existing conditions with increased frequency or severity or newly developed conditions.
    Please describe.

14. (To be asked of home gutting laborers) Would you mind if we contact you in the future to follow up on your health status ?   Feel free to contact me_____   Do not contact me_____

Name: _____   Phone:

_____

Address:

_____

Email: _____

Fulfillment of Prospectus Defense Addendum:

In order to strengthen my knowledge of Immunology and Microbiology and apply the acquired information to my research, I utilized course information from Massachusetts' Institute of Technology's (MIT) graduate level "Cellular and Molecular Immunology" Open Courseware. The course (HST.176) was taught by Dr. Shiv Pillai, and lecture notes were located at: http://ocw.mit.edu/courses/health-sciences-and-technology/hst-176-cellular-and-molecular-immunology-fall-2005/. Additional resources used in the completion of the research can be found in the "Works Cited" list.

Leaf Surface, Soil and Water-Indicator Fungi in Our Research

The following fungal taxa, categorized according to ecological groups, were analyzed in air, dust and/or blood samples in this dissertation research. Fungi were classified based on information adapted from Horner et al's characterization of fungal groups in non-water-damaged Atlanta homes (the study generalized results to include most southeastern homes), an overview of indoor airborne fungi by Levetin and descriptions of fungal taxa by Dobranic, EMLab and Phadia (Dobranic 2004; EMLab P&K 2011; Horner et al 2004; Levetin *n.d.*, Phadia 2011). According to Horner et al, leaf surface fungi should be predominant in indoor samples and should account for at least 20 - 80% of the fungal composition in most non-water damaged dust samples; additionally, microbial composition in indoor vs. outdoor air should not greatly differ (2004). The rationale for dust in the Horner et al study has been generalized to include the leaf surface ratios for our study's air sample as a means of comparing microbial composition.

- ❖ **Leaf Surface Fungi** (also called phylloplane, are commonly present on the surfaces or undersides of vegetation and may be saprobic):
 - o *Alternaria*
  - o *Aureobasidium pullulans (black yeast)*
  - o *Cercospora*
  - o *Cladosporium*
  - o *Curvularia*
  - o *Epicoccum*
  - o *Nigrospora*

- o *Torula*

❖ **Soil Fungi** (often found in the soil and may also act as plant pathogens):

- o *Aspergillus* spp. (includes A. flavus, *A. fumigatus*, *A. niger* and *A. versicolor*)- sexual spore form: *Emericella*

- o *Fusarium*

- o *Mucor*

- o *Penicillium*

- o *Paecilomyces*

- o *Pithomyces*

- o *Stemphylium* (similar to *Sporidesmium*, although the former has conidia connected to the conidiophores only by a thread) (Moore 1958)

- o *Syncephalastrum*

- o *Verticillium*

- o zygomycetes (*Zygosporium*)

❖ **Water-Indicator Fungi** (often detected on materials in water-damaged buildings; all listed are soil fungi):

- o *Chaetomium globosum*

- o *Stachybotrys chartarum*

- o *Trichoderma viride*

❖ **Other groups:**

- o ascospores: sexual spores produced in an ascus (sac); large group which includes spores from most fungal genera

- basidiospores: sexual spores produced from a basidium; includes mushrooms (*Ganoderma* is one genus of mushroom that may have medicinal uses) and is commonly associated with dry rot in buildings; soil fungi

- *Bipolaris/Drechslera*: a group of similar fungal genera comprised of *Bipolaris* (soil), *Drechslera* (leaf surface) *and Helminthosporium*

- Non-sporulating: spores that cannot be identified since they require specialized environmental conditions to grow

- *Smuts/Periconia/Myxomycetes* (soil): group of spores that are difficult to distinguish microscopically when collected in non-viable air sampling (all have round, brown spores); smuts and myxomycetes are not true fungi based on taxonomy (they are mobile like amoeba under certain conditions)

Late post-Katrina research photos

Pre-gutting photos of Non-renovated Home 2 (NRH2) - "Fungal Allergen-Mold Biomarker" manuscript (#3) - 06/01/2009
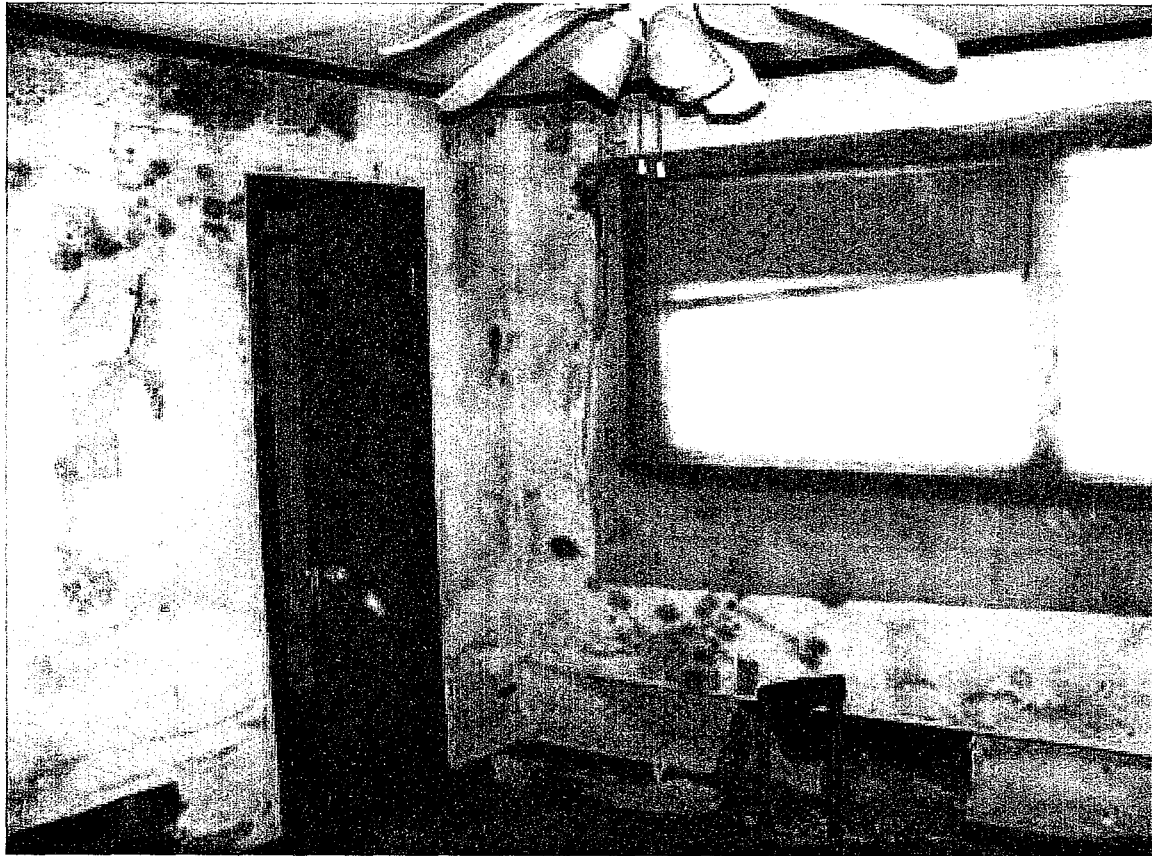
Master bedroom-sampling site

Pre-gutting photos for NRH2 cont.- Bulk composite dust samples from the kitchen and the living room for "Fungal and

Bacterial Markers" manuscript (#2) - also analyzed dust for *A. versicolor* and *S. chartarum* antigen with ELISA.
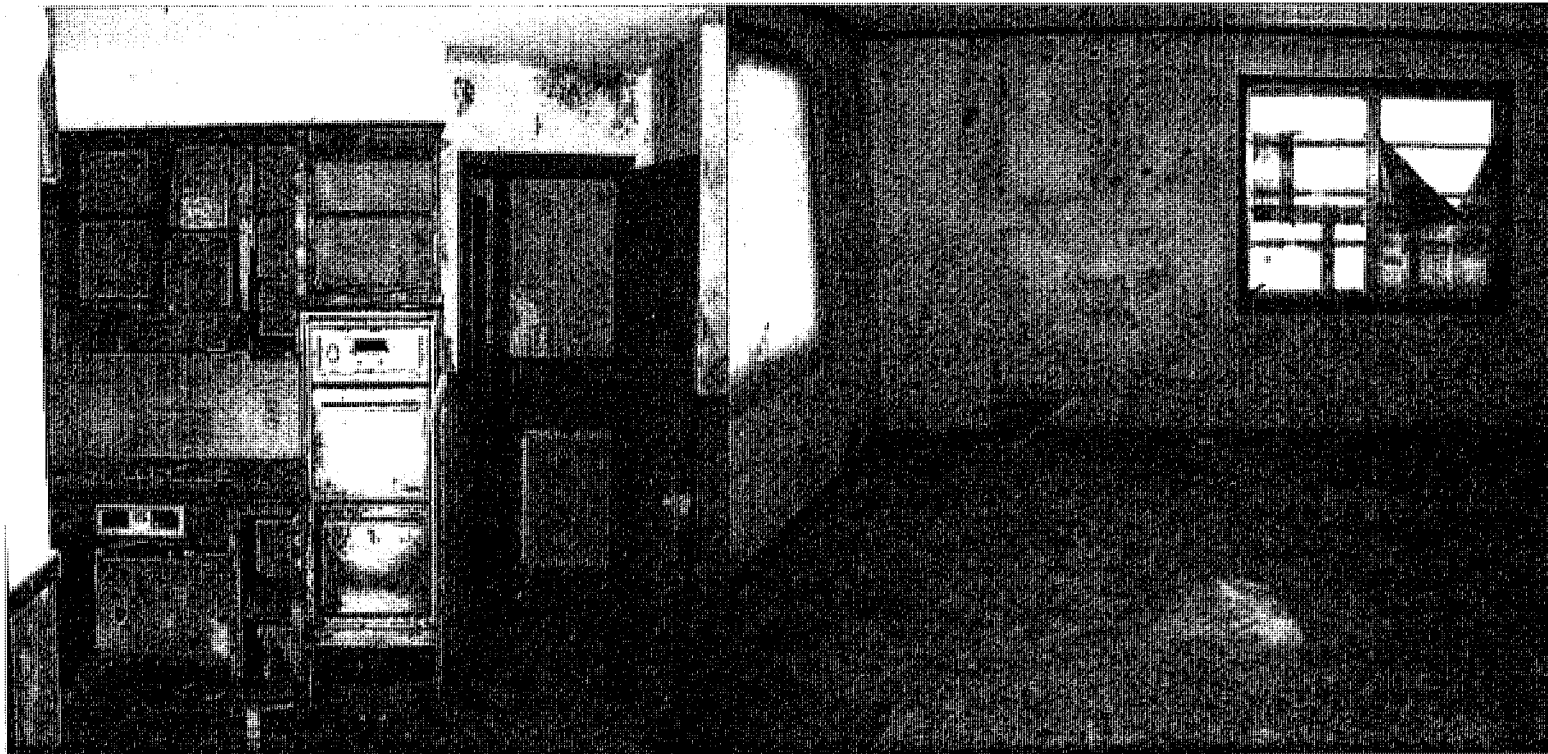
Kitchen   Living Room

Post-gutting photos for Non-renovated Home 2/ Partially renovated home (PRH)

| Bedroom photo 1 | Bedroom photo 2 | Living room |

Bedroom- sampling site

Appendix C- Published Co-Authored Articles

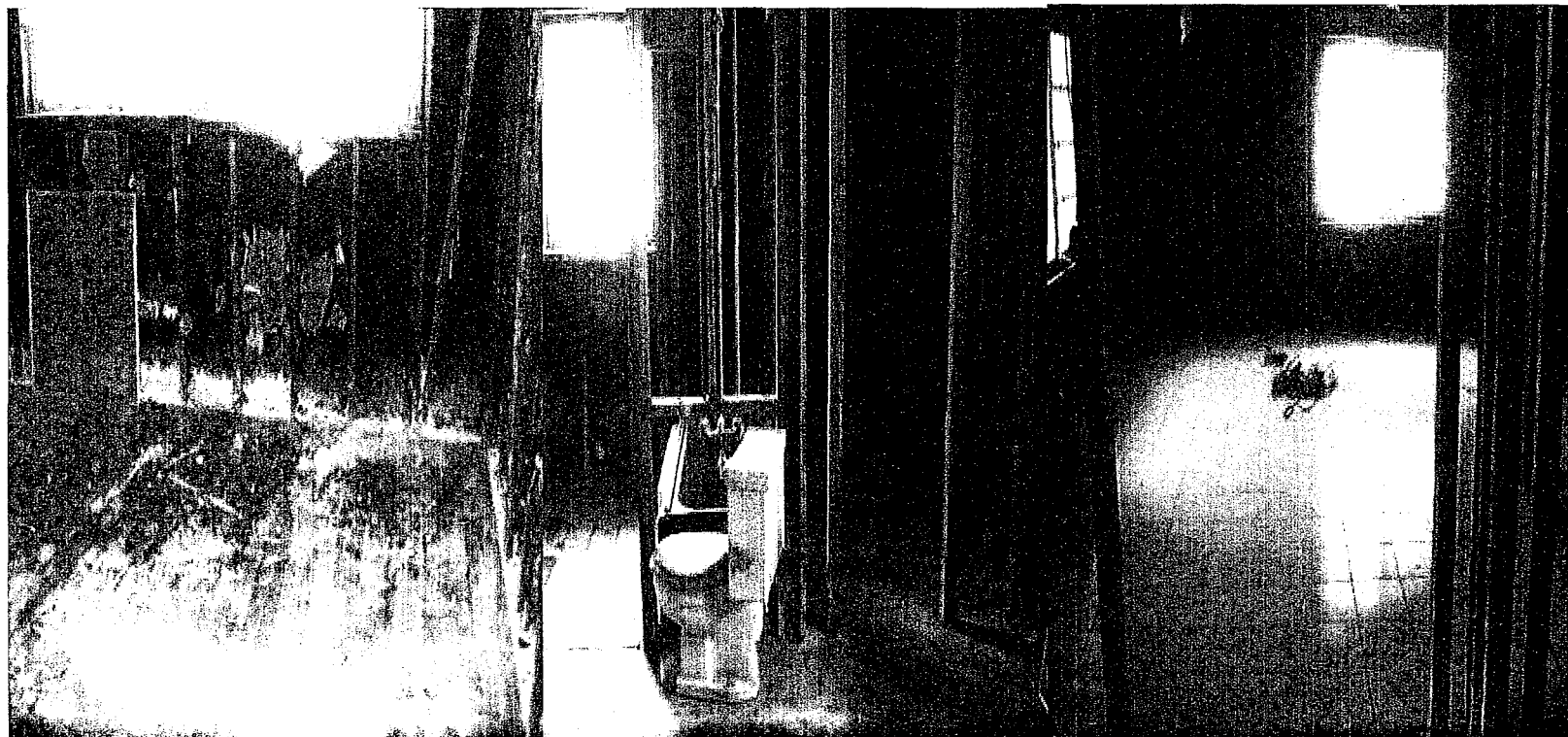1) Adhikari A, Jung J, Reponen T, **Lewis JS**, DeGrasse EC, Grimsley LF, Chew GL, Grinshpun SA. Aerosolization of fungi, (1-->3)-beta-D glucan, and endotoxin from flood-affected materials collected in New Orleans homes. Environ Res 2009;109: 215-24. http://www.ncbi.nlm.nih.gov/pubmed/19201399.

2) Adhikari A, **Lewis JS**, Reponen T, DeGrasse EC, Grimsley LF, Chew GL, Iossifova Y, Grinshpun SA. Exposure matrices of endotoxin, $(1->3)-\beta-d$-glucan, fungi and dust mite allergens in flood-affected homes of New Orleans. Sci Total Environ 2010;408:5489-98. http://www.ncbi.nlm.nih.gov/pubmed/20800874.

3) Bloom E, Grimsley LF, Pehrson C, **Lewis J**, Larsson L. Molds and mycotoxins in dust from water-damaged homes in New Orleans after hurricane Katrina. Indoor Air 2009;19:153-8. http://www.ncbi.nlm.nih.gov/pubmed/19191.

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Contents lists available at ScienceDirect

Environmental Research

Aerosolization of fungi, (1→3)-β-D glucan, and endotoxin from flood-affected materials collected in New Orleans homes ☆

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ARTICLE INFO

ABSTRACT

Standing water and sediments remaining on flood-affected materials were the breeding ground for many microorganisms in flooded homes following Hurricane Katrina. The purpose of this laboratory study was to examine the aerosolization of culturable and total fungi, (1→3)-β-D glucan, and endotoxin from eight flood-affected floor and bedding materials collected in New Orleans homes, following Hurricane Katrina. Aerosolization was examined using the Fungal Spore Source Strength Tester (FSSST) connected to a BioSampler. Dust samples were collected by vacuuming. A two-stage cyclone sampler was used for size-selective analysis of aerosolized glucan and endotoxin. On average, levels of culturable fungi ranged from undetectable (lower limit = 8.3×10^4) to 2.6×10^5 CFU/m^2; total fungi ranged from 2.07×10^5 to 1.6×10^6 spores/m^2; (1→3)-β-D glucan and endotoxin were 2.0×10^3–2.9×10^4 ng/m^2 and 7.0×10^2–9.3×10^4 EU/m^2, respectively. The results showed that 5–15 min sampling is sufficient for detecting aerosolizable biocontaminants with the FSSST. Smaller particle size fractions (<1.0 and <1.8 μm) have levels of glucan and endotoxin comparable to larger (>1.8 μm) fractions, which raises additional exposure concerns. Vacuuming was found to overestimate inhalation exposure risks by a factor of approximately 10^2 for (1→3)-β-D glucan and by 10^3–10^4 for endotoxin as detected by the FSSST. The information generated from this study is important with respect to restoration and rejuvenation of the flood-affected areas in New Orleans. We believe the findings will be significant during similar disasters in other regions of the world including major coastal floods from tsunamis.

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1. Introduction

During last decade many countries of the world have seen natural disasters from floods. The unprecedented disasters caused by Hurricanes Katrina and Rita in 2005 disrupted the public health and medical infrastructures in New Orleans and created many difficult environmental health challenges. Standing water and sediments remaining in flooded areas were breeding grounds for various microorganisms, including fungi and bacteria that could become airborne and be inhaled; in turn, these exposures may increase the incidence of lung disease (American Lung Association, 2008), among other health effects. Fungi have been

associated with allergic respiratory disease, especially asthma (Douwes et al., 2003). Total fungi, including spores from both culturable and non-culturable species, may be as allergenic or toxigenic as the culturable fungi (Flannigan, 1997). Viable fungi can release allergens during germination (Green et al., 2003) and may cause mycotic infections in immunocompromised subjects (Burge, 2001; Eduard, 2003). The association of (1→3)-β-D glucan (a polyglucose molecule comprising up to 60% of the cell wall of most fungal taxa) with dry cough, phlegmy cough, hoarseness, and atopy has been reported in indoor environments (Rylander et al., 1998; Rylander, 1999). Bacterial growth in flooded homes can be a significant source of endotoxin, which is a lipopolysaccharide component of the outer membrane of Gram-negative bacteria. Inhaled endotoxin can contribute significantly to the induction of airway inflammation and dysfunction (Pirie et al., 2003).

There are significant public health concerns about exposure to airborne microorganisms and the associated respiratory health effects; however, the scientific knowledge regarding the aerosolization of microorganisms in field conditions, including the

flood-affected buildings, is still insufficient. While air sampling is currently deemed to be the most representative method of determining respiratory exposure, there are several problems with traditional air sampling methods. Most commercially-available bioaerosol sampling instruments (e.g., Burkard, Andersen, Air-O-Cell) cannot be used for assessing long-term exposure to airborne microorganisms, as their sampling time is limited to 3–20 min. At the same time, the spore concentrations have wide temporal variations, particularly in mold-contaminated homes (Hyvärinen et al., 2001). In addition, each sampling method has limitations, and a standard unanimously-accepted bioaerosol sampling method is not yet available.

Information about airborne microorganisms that is assessed by conventional air sampling during specific time intervals may not be representative of potential exposure levels; it may not be able to reliably detect microbial colonization and their successive aerosolization from contaminated surfaces (Horner, 2003). Moreover, the release of microorganisms from surfaces does not necessarily occur during air sampling. Release of certain microorganisms may be sporadic, even with no disturbance of the surfaces and especially when it is caused by movements of occupants or workers engaged in clean-up activities. It has been widely recognized that a reliable technique is required to directly assess the aerosolization potential of microorganisms growing on contaminated surfaces. Direct source evaluation techniques, such as bulk sampling, surface sampling (e.g., swab and tape sampling) and dust sampling by vacuuming, allow the investigators to collect extensive information about the source. However, the aerosolization potential of contaminants from their sources cannot be measured with these methods, as they collect both aerosolizable contaminants as well as those that adhere to the deeper surface of substrates; thus, they are unlikely to become aerosolized under normal circumstances. Therefore, the results may not adequately represent or predict the exposure risks to aerosolized microorganisms from a contaminated material. The problem has been addressed in this study by utilizing a Fungal Spore Source Strength Tester (FSSST), an inexpensive and portable device previously developed and evaluated by our group. The device was found suitable for aggressive sampling of releasing potentially aerosolizable fungal spores from mold-contaminated sources (Grinshpun et al., 2002; Sivasubramani et al., 2004a,b; Niemeier et al., 2006; Seo et al., 2008). This study utilized the FSSST to gain a fundamental understanding of the aerosolization of the potential moisture-related microbiological hazardous agents (culturable and total fungi, $(1\rightarrow3)$-β-D glucan, and endotoxin).

In addition to these three biocontaminants, dust mite allergens were also analyzed in selected samples. There were two reasons behind these tests. First, we anticipated that lower levels of aerosolized fungi, observed for several materials, can be influenced by the presence of dust mites, which affect the fungal growth surfaces. Second, dust mites can use fungal mycelium as food, thus facilitating fragmentation and subsequent aerosolization of small particles containing relatively higher amount of $(1\rightarrow3)$-β-D glucan. There are reports of interactions between domestic dust mites and fungi in indoor environments (Asselt, 1999) and antagonistic and mutual ecological relationships between xerophilic fungi (found prevalent in this study) and dust mites at higher relative humidity (Lustgraaf, 1978).

Another important focus in this study was to determine the concentrations of aerosolized biocontaminants size-selectively. Bioaerosol particles vary in size depending upon the microorganism, the aerosolization mechanism, and environmental parameters (Reponen et al., 1994, 1996; Meklin et al., 2002; Górny et al., 2002; Lindsley et al., 2006; Wang et al., 2007). Furthermore, fine particles may be more damaging to human respiratory health

than larger ones, regardless of their low mass, as they penetrate deeper into airways (Ferin et al., 1990; Oberdorster, 1996). The National Institute for Occupational Safety and Health (NIOSH) has recently developed a two-stage cyclone sampler for size-selective personal sampling of bioaerosols (Lindsley et al., 2006). In this study, this sampler was used to investigate aerosolized $(1\rightarrow3)$-β-D glucan and endotoxin in three size fractions.

To summarize, the purposes of this laboratory study were: (i) to determine aerosolization of culturable and total fungi, $(1\rightarrow3)$-β-D glucan, and endotoxin from eight different flood-affected materials; (ii) to investigate aerosolization of $(1\rightarrow3)$-β-D glucan and endotoxin with size-selective sampling; and (iii) to compare the levels of these biocontaminants measured by FSSST and a vacuum cleaner.

2. Materials and methods

2.1 Collection of flood-affected materials

Six floor materials (linoleum, a small rug, a water-affected small rug, an area rug, a thick carpet, and a thin carpet) and two bedding materials (a pillow and a mattress), each at least $0.25\,m^2$, were collected from six flood-affected homes in New Orleans. The homes with at least ~3 ft flood water level were selected and six different types of materials were randomly collected to accommodate typical floor and bedding materials commonly used in a household. Homes were not renovated and ceiling and wall materials were excluded because they were not readily available from the owners. The materials were collected approximately 1 year after Hurricane Katrina, however, they were determined to have moisture incursion and thus were expected to have fungal and bacterial contamination. Environmental characteristics of these homes are summarized in Table 1. Two types of indoor carpets, with considerably different fiber lengths, were collected. The thicknesses of the thick and thin carpets were 15 and 9 mm, respectively, and both were made of synthetic fibers. The water-affected small rug and the mattress had some moisture at the time of collection and upon conduction of the experiments. Other materials were also affected by flood water; however, they were dry and covered with sediment and dust at the time of collection.

The material samples were collected in clean airtight plastic bags after cutting from the surfaces (if applicable) with appropriate biosafety precautions. Immediately after collection from homes, the samples were sent to the University of Cincinnati and were preserved in laboratory incubators at approximately 25 °C and 51–72% RH until experiments could be conducted (2–3 weeks post-collection). It was decided to store the material samples at room temperature to avoid moisture condensation on the material surfaces, as this could affect natural aerosolization potential of biocontaminants.

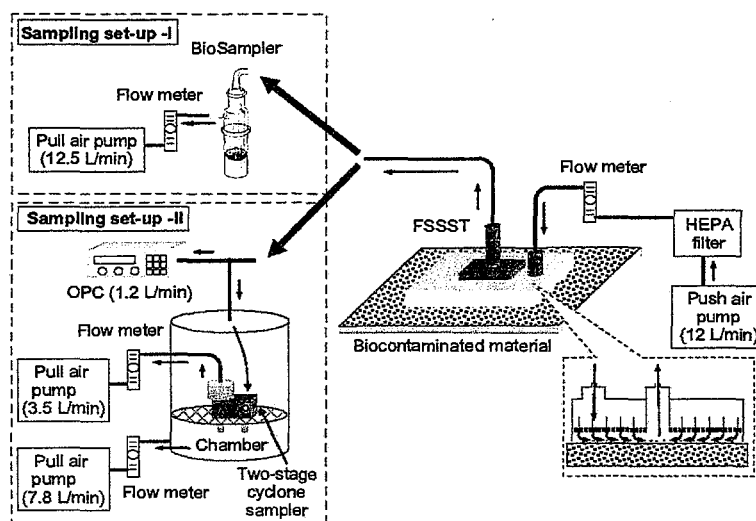### 2.2 Study of the aerosolization of biocontaminants from flood-affected materials via FSSST

As stated above, the aerosolization of viable and total fungi, $(1\rightarrow3)$-β-D glucan, and endotoxin were studied using the FSSST. The FSSST sampling unit is a closed aerosolization chamber, equipped with two pumps, that was secured on the flood-affected contaminated material surface during aerosolization tests. A push vacuum pump produces the airflow that passes through the HEPA filter (1244 HEPA capsule filter; PALL Gelman Laboratory, Ann Arbor, MI, USA). The incoming air flow Q_{IN} (12 L/min), directed through the 112-orifice stage at the bottom of the device, creates air jets toward the contaminated surface; the biocontaminants are aerosolized by the air jets and were collected into a BioSampler (SKC, Inc., Eighty Four, PA, USA) (see sampling set-up I in Fig. 1) connected with the FSSST set-up through a vinyl tubing. The process inside the FSSST cap simulates the release of biocontaminants from the surface, where they have been accumulate, with their subsequent (immediate) collection into the BioSampler. The detailed design and performance characteristics of the FSSST have been presented elsewhere (Grinshpun et al., 2002; Sivasubramani et al., 2004a; Górny et al., 2002).

The BioSampler was located at the outlet of the FSSST and operated by a pull vacuum pump at $Q_{SAMPLE} = 12.5$ L/min. The collection vessel of the BioSampler was filled with a 20 mL suspension of pyrogen free, sterilized water mixed with 0.05% Tween 80. To minimize contamination of ambient air, the flow rate balance was set so that Q_{IN} was slightly lower than Q_{SAMPLE} ($\Delta = 0.5$ L/min). The device was thoroughly cleaned between the tests with 70% ethyl alcohol, and a separate sterile BioSampler was used for each test. After sampling, the collection fluid from each BioSampler was divided into aliquots. One aliquot was immediately used for culturing fungi, and the other aliquots were stored at −20 °C for the analyses of $(1\rightarrow3)$-β-D-glucan, endotoxin, and total fungi (by microscopy).

All aerosolization tests were conducted inside a Class II Biosafety cabinet. Three replicate samples were collected from different areas of each material

Table 1
Characteristics of flood-affected homes and locations from where eight floor and bedding materials were collected.

Material	Home type	Room type	Flood-water level (feet)	Temperature (°C)	Relative humidity (%)	Visible mold
Linoleum and small rug	Wood frame and hardwood floor	Living room and kitchen	~3	23.8	54.2	Only linoleum had several dry spots
Small rug (water affected) and pillow	Wood frame and hardwood floor	Living room and kitchen	~3	21.8	67.8	No
Area rug	Brick ranch home on slab; floor with hardwood, tile, terrazzo, and area rug	Central living area	~10	10.5	34	Several dry spots near the margins
Thick and thin carpets	Raised double (shotgun style); wood flooring in bedrooms, carpet in living room and master bed room	Living room	~5	20.0	31	No
Mattress	Raised double (shotgun style) with wood frame, wood flooring throughout with tile in kitchen and bathroom	Bedroom	~10	22.7	46	A few black spots indicating active mold growth

Abbreviations: OPC = Optical particle counter; FSSST: Fungal Spore Source Strength Tester; HEPA filter: High efficiency particulate air filter.

Fig. 1. Experimental set-up to test the aerosolization of biocontaminants (set-up I) and size-selective measurement of aerosolized biocontaminants (set-up II).

(randomly selected approximately 0.25 m² of the surface area), and appropriate blanks (samples from sterile aluminum foil surfaces) were collected as well. Each replicate consisted of samples collected for 5, 10, and 20 min consecutively from the same sampling spot and cumulative biocontaminant levels of 5, 15 (= 5+10), and 35 (= 5+10+20) min were used for data analysis.

Bioaerosol concentrations are normally expressed as an amount per cubic meter of air. However, in this study, to represent aerosolizable biocontaminants, their amounts were expressed per square meter of the area from which they were aerosolized. This allowed for the generation of the data that can be compared to those obtained from vacuum samples. By characterizing the release rate, one can calculate the concentration of the biocontaminants applying specific air volume and ventilation rate for the room. These results should be considered as the maximum aerosolizable concentration level of each selected biocontaminant in indoor air.

2.3. Collection of culturable fungi from material surfaces by swabbing and extraction

For comparison, two additional methods were also applied for the analysis of culturable fungi. The first was swab sampling of 1 cm² surfaces with wetted (using sterile distilled water) sterile cotton wood-tipped sticks, following the protocol described by Hung et al. (2005). The second was extraction of 1 cm² pieces of

contaminated materials in 10 mL de-ionized sterile water with 0.05% Tween 80 using a touch mixer (model 231, Fisher Scientific, Pittsburgh, PA, USA) for 2 min and ultrasonic bath (FS20, Fisher Scientific) agitation for 10 min. The water in the ultrasonic bath was replaced between sonication of individual samples to avoid the temperature increase.

2.4. Particle size-selective study of (1→3)-β-D glucan and endotoxin using the NIOSH two-stage cyclone sampler combined with FSSST

The NIOSH two-stage cyclone sampler consists of two screw-top 1.5 mL microcentrifuge tubes (Model no. 506-624: PGC Scientifics Corp., Frederick, MD) and a 37-mm filter holder with 0.8 μm polycarbonate filter (SKC Inc.) (see sampling set-up II in Fig. 1) connected with the FSSST set-up through a vinyl tubing. At an air flow rate of 3.5 L/min, the 50% cut-off diameters of the first and second tubes are 1.8 and 1.0 μm, respectively. Thus, the sampler collects aerosolized biocontaminants into three particle size ranges: <1.0, 1.0–1.8, and >1.8 μm. An Optical Particle Counter, OPC (model 1.108, Grimm Technologies, Inc., Douglasville, GA, USA) with a flow rate of 1.2 L/min was operated in parallel to the cyclone sampler to monitor the release stability of aerosolized biocontaminants. The design and performance characteristics of the NIOSH two-stage cyclone sampler were

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presented by Lindsley et al. (2006). The size-selective samples were analyzed for (1→3)-β-D glucan and endotoxin content for five selected material samples.

2.5. Collection of dust samples by a vacuum cleaner

Dust samples were collected from the same eight collected materials, using a standardized dust sampling protocol (HUD, 2004), into a small filter bag by vacuuming approximately 0.25 m² (adjacent to positions used for FSSST) with a Filter Queen Majestic® vacuum cleaner (Health-Mor, HMI Industries Inc., Seven Hills, OH, USA) for 5 min. Samples were collected on the same day followed by the experiments with FSSST. Dust samples were sieved (355 μm sieve; No. 45; W.S. Tyler, Mentor, OH, USA); the fine dust was divided into sub-samples and stored at −20 °C until the analysis. Immediately before analysis, aliquots of dust were extracted for (1→3)-β-D-glucan and endotoxin, as described below. For dust mite allergen extraction, one aliquot of 100 mg was extracted in 2 mL phosphate-buffered saline (PBS) with 0.05% Tween 20, and incubated at 30 °C on a platform shaker for 1 h. Dust samples were not used for fungal analysis, as spores are masked by dust particles in microscopic spore counting, and preliminary data showed levels of viable fungi below the lower limit of detection (<LOD) in many samples.

2.6. Analysis of culturable fungi

Culture-based analyses were performed for all of the samples collected with the Biosampler, as well as for swab samples and samples extracted directly from selected materials. The extracts were serially diluted and plated on 2% malt extract agar (MEA; Difco Laboratories, Detroit, MI, USA) and dichloran-18-glycerol agar (DG18; Oxoid, Basingstoke, Hampshire, UK) with chloramphenicol antibiotic at a concentration of 100 mg/L. The plates were incubated at 25±2 °C for 7 days. Blank media and blank buffer cultivations were also conducted for quality control. Colonies were identified to genus, based on gross morphology and spore-forming structures; high-resolution light microscopy was used (Labophot 2, Nikon Corp., Tokyo, Japan), following the illustrated identification manuals by Smith (1990) and Ellis (1971) and based on reference slides (Aerobiology Instruction and Research, Brookline, MA, USA). The confirmed colonies were enumerated, and concentrations were expressed as aerosolized CFU per m² of contaminated surfaces.

2.7. Microscopic analysis of total fungi

An aliquot of the BioSampler extract was filtered through a 13-mm mixed cellulose esterase filter (Millipore Corp., Bedford, MA, USA). Each filter was placed onto a slide and allowed to completely dry. Once the filter was dry, the filter was made clear through treatment with acetone vapor, as described by Adhikari et al. (2003). Fungal spores in all air samples were counted on the filter in 40 microscopic fields using a bright light microscope (Labophot2, Nikon Corp., Japan) at a magnification of 400× or 1000×, if required. Fungal spores were identified morphologically to genus/group level (following the same manuals and reference slides stated above), and the results were expressed as aerosolized spores/m².

2.8. Analysis of (1→3)-β-D glucan

The (1→3)-β-D-glucan concentrations in different extracts were analyzed using the kinetic chromogenic Limulus Amebocyte Lysate (LAL) assay (Glucatell, Associates of Cape Cod, East Falmouth, MA, USA). This assay has been successfully used for analysis of (1→3)-β-D-glucan, in both air and dust samples by the University of Cincinnati group (Lee et al., 2006; Iossifova et al., 2007). Protocols used in the referenced investigations were followed in the present study. The Biosampler suspension of 0.5 mL was treated with 0.5 mL of 0.6 M NaOH and extracted for 1 h. For dust samples, 25 mg dust was extracted in 1.0 mL of 0.6 M NaOH for 1 h, followed by centrifugation at 7000 rpm (5204g) for 1 min. The supernatant liquid extracts were used for (1→3)-β-D-glucan analysis. Aerosolized (1→3)-β-D-glucan concentrations were presented in ng/m².

2.9. Analysis of endotoxin

Endotoxins in different extracts were analyzed using the endotoxin-specific kinetic chromogenic LAL-assay (Pyrochrome, Associates of Cape Cod). In total 0.5 mL of liquid extracts from BioSamplers were sonicated for 1 h at room temperature, with shaking for 15 s every 15 min using a touch mixer (Fisher Scientific). For dust samples, 25 mg of dust was extracted in 1.0 mL pyrogen-free sterile water for 1 h by sonication and intermittent shaking, followed by centrifugation at 7000 rpm for 1 min. Supernatants were collected and analyzed as stated above. Endotoxin results were presented in EU/m².

2.10. Analysis of dust mite allergens

Dust extracts were serially diluted in phosphate-buffered saline with 1% bovine serum albumin and 0.05% Tween 20 (BSA-PBS-T) at pH 7.4. Two-site monoclonal antibody (MAB) sandwich ELISAs for Der p 1 and Der f 1 were used (Indoor Biotechnologies, Inc., Charlottesville, VA, USA) as described by Luczynska et al. (1989). The LOD for both Der p 1 and Der f 1 analyses by this method was 0.025 μg/g.

2.11. Statistical analysis

Normal distributions of the data were checked by Shapiro–Wilk and Kolmogorov–Smirnov tests. Analysis of variance (ANOVA) and Kruskal–Wallis tests were conducted to compare normally-distributed and non-normally distributed data, respectively, for different collection times and size fractions. Scheffe and Bonferroni post-hoc tests were conducted to understand which groups differ for ANOVA and Kruskal–Wallis tests (at $P = 0.05$ level of significance), respectively. Non-parametric Spearman's correlation coefficients were calculated to check the correlation between the aerosolized (1→3)-β-D-glucan and endotoxin levels, when the data were not normally distributed. Values <LOD were replaced by LOD/2 in the statistical analyses. All statistical tests were performed using the SPSS 11.0 for Windows (SPSS Inc., Chicago, IL, USA) and SAS/Stat 9.1 (SAS Institute Inc., Cary, NC, USA) softwares.

3. Results

3.1. Aerosolization of culturable fungi

As presented in Table 2, the average levels of culturable fungi aerosolized from different contaminated materials ranged from <LOD to approximately 2.59×10^5 CFU/m². The highest culturable fungi concentration was observed when examining aerosolization from the contaminated mattress, which was slightly moist at the time of collection. Colonies of Aspergillus, Penicillium, and Cladosporium, along with non-sporulating colonies were detected in different samples.

With the linoleum, the small rug, and the pillow excluded (as their levels of aerosolized culturable fungi were <LOD), the culturability ranged from 3% (area rug) to 41% (mattress) [culturability % = concentration of culturable fungi/concentration of total fungi × 100]. Since the colony counts were <LOD in many aerosol samples, culturable analysis was also performed for fungi collected by swabbing and extraction from materials such as linoleum and mattress. These samples represented extremes with respect to the levels of aerosolized culturable fungi. For the mattress, swabbing provided a similar concentration (1.6×10^5 CFU/m²) of viable fungi to that determined with the FSSST, while extraction provided an approximately 10-fold higher concentration (2.0×10^6 CFU/m²). Extraction and swabbing from linoleum provided culturable fungi concentrations of 5.0×10^5 and 4.0×10^4 CFU/m², respectively.

3.2. Aerosolization of total fungi

Since a considerable number of samples exhibited low levels (<LOD) of aerosolized culturable fungi, only 33% of the FSSST samples (one sample out of every three replicates for each material was randomly chosen) were analyzed for total fungi. The results on aerosolized total fungi (a cumulative level for 35 min) and individual genera/classes are presented in Tables 2 and 3. In addition to the genera presented in Table 3, we found sporadic presence of Arthrinium, Ganoderma, Polythrincium, and Torula spores. The concentrations of total aerosolized fungi in different materials ranged approximately from 2.07×10^5 to 1.6×10^6 spores/m².

When log-transformed cumulative levels of total aerosolized fungi for 8 materials collected during consecutive 5, 10, and 20 min (cumulative 5, 15, and 35 min) were compared using

Table 2

Biocontaminants aerosolized from floor and bedding materials collected in flood-affected homes of New Orleans using FSSST (cumulative levels over 35 min).

Material	Culturable fungi ($\times 10^3$ CFU/m^2)	Total fungi[a] ($\times 10^3$ spores/m^2)	(1→3)-β-D glucan ($\times 10^3$ ng/m^2)	Endotoxin ($\times 10^3$ EU/m^2)
Linoleum	<LOD	475	18.25±23.57	59.15±70.45
Small rug	<LOD	259	28.54±32.94	93.09±141.52
Small rug (water affected)	8±8	615	21.56±26.45	9.86±4.16
Pillow	<LOD	710	13.12±8.90	8.39±4.91
Area rug	25±22	1145	12.18±7.40	37.37±30.28
Thick carpet	8±8	207	3.43±1.90	0.70±0.58
Thin carpet	11±19	324	2.00±2.26	0.81±0.31
Mattress	259±353	1598	2.01±0.64	11.52±4.81

Note: values represent cumulative data over three consecutive sampling time periods of 5, 10, and 20 min ($t = 35$ min). Three replicates ($n = 3$) were averaged and mean±SD data are presented, except for total fungi. <LOD = no detectable CFUs in the sample.
Lower limit of detections (LOD): culturable fungi: 8264 CFU/m^2; total fungi: 5587 spores/m^2; (1→3)-β-D-glucan: 8.39 ng/m^2; endotoxin: 86.76 EU/m^2.
[a] Total fungi were analyzed in 33% samples only ($n = 1$).

Table 3

Composition of fungal spores aerosolized from floor and bedding materials collected in flood-affected homes of New Orleans ($\times 10^3$ spores/m^2).

Material	Linoleum	Small rug	Small rug (water-affected)	Pillow	Area rug	Thick carpet	Thin carpet	Mattress
Fungal genera/class								
Alternaria	6	6	6	6	<LOD	<LOD	<LOD	<LOD
Ascospores	17	<LOD	39	39	28	11	6	<LOD
Aspergillus/Penicillium	369	199	520	564	927	151	212	469
Basidiospores	<LOD	11	6	11	<LOD	<LOD	<LOD	<LOD
Botrytis	11	6	6	<LOD	6	<LOD	<LOD	<LOD
Chaetomium	<LOD	6	<LOD	<LOD	<LOD	<LOD	<LOD	61
Cladosporium	34	<LOD	22	28	45	<LOD	6	<LOD
Stachybotrys	<LOD	6	6	<LOD	39	6	22	1034
Unknown	39	22	11	61	101	39	61	34
Total spores	475	260	615	710	1145	207	324	1598

one-way Analysis of variance (ANOVA) in all 8 materials, a significant difference was found ($P<0.05$) between sampling periods. Scheffe post hoc test, however, showed a difference only between the levels of total aerosolized fungi collected at 5 and 35 min.

3.3. Aerosolization of (1→3)-β-D glucan

The range of (1→3)-β-D glucan aerosolized from different materials ranged approximately from 2.0×10^3 to 2.9×10^4 ng/m^2 (mean values, Table 2). When aerosolization for cumulative periods of 5, 15, and 35 min were compared, there was a slight increase of aerosolization with time (Fig. 2). However, this was not statistically significant in most samples ($P>0.05$ in ANOVA and Kruskal–Wallis tests). Only for the thick carpet was a significant difference observed, and post hoc multiple comparisons indicated that the concentration at 5 min differed significantly from the concentration at 35 min. A large variation in the aerosolized (1→3)-β-D glucan levels was observed for the three replicate samples collected from different positions from the same materials [coefficient of variation (CV) = 60–129%].

Size-selective data on (1→3)-β-D glucans aerosolized from different materials are presented in Fig. 3. There were no significant differences among the size fractions of <1.0, 1.0–1.8, and >1.8 μm for three of the five tested materials ($P>0.05$; ANOVA and Kruskal–Wallis tests). In the case of the linoleum and the mattress, a significant difference was observed ($P<0.05$; Kruskal–Wallis tests). The Bonferroni post-hoc test revealed that the size fraction >1.8 μm was different from the size fraction of <1 μm for linoleum and from the size fraction of 1–1.8 μm for mattress.

3.4. Aerosolization of endotoxin

Endotoxin aerosolized from different materials ranged from 7.0×10^2 to 9.3×10^4 EU/m^2 (mean values, Table 2). There was a slight increase of aerosolization from all materials with time (Fig. 4); however, the differences were not statistically significant ($P>0.05$; ANOVA and Kruskal–Wallis tests). Similar to (1→3)-β-D glucan, large variations of aerosolized endotoxins were observed in samples collected from different positions of the same material (CV = 38–119%).

The size-selective data on the aerosolized endotoxin are presented in Fig. 5. Higher concentrations were observed for the larger fraction (>1.8 μm) than for the other two; however, no statistically significant differences were found.

3.5. Correlation between aerosolized (1→3)-β-D glucan and endotoxin

Non-parametric Spearman's correlation coefficient between the data on aerosolized (1→3)-β-D glucan and endotoxin collected from all materials were calculated for all three sampling periods. A significant positive correlation was observed between the two data sets (correlation coefficient = 0.425; $P<0.001$).

3.6. Surface (1→3)-β-D glucan and endotoxin investigated with a vacuum cleaner

In all cases, except for the pillow, the vacuum cleaner sample showed higher levels of (1→3)-β-D glucan and endotoxin than the FSSST (Table 4). The average ratios (vacuum sample to FSSST sample) were 113±132 for the glucan and 4594±8022 for the endotoxin (mean±SD). The levels of (1→3)-β-D glucan and

Fig. 2. $(1\rightarrow3)$-β-D-glucan aerosolized from contaminated materials collected in flood-affected homes of New Orleans. The histograms present geometric mean of three replicates and error bars indicate geometric standard deviations.
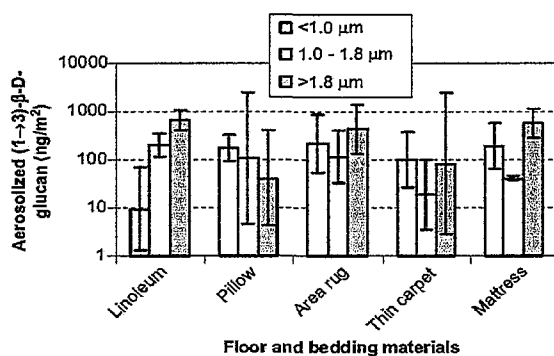
Fig. 3. $(1\rightarrow3)$-β-D-glucan aerosolized from different floor and bedding materials collected in flood-affected homes of New Orleans (presented size-selectively). The histograms present geometric mean of three replicates and error bars indicate geometric standard deviations.
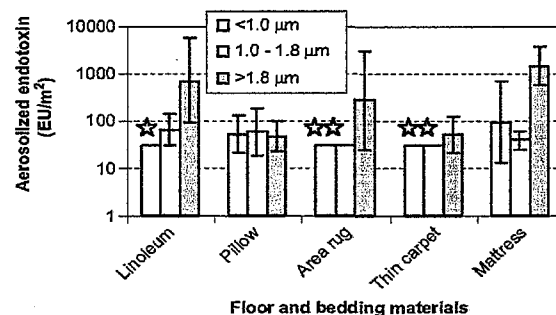
Fig. 5. Endotoxin aerosolized from different floor and bedding materials collected in flood-affected homes of New Orleans (presented size-selectively). The histograms present geometric mean of three replicates and error bars indicate geometric standard deviations. Star signs indicate <LOD data points.
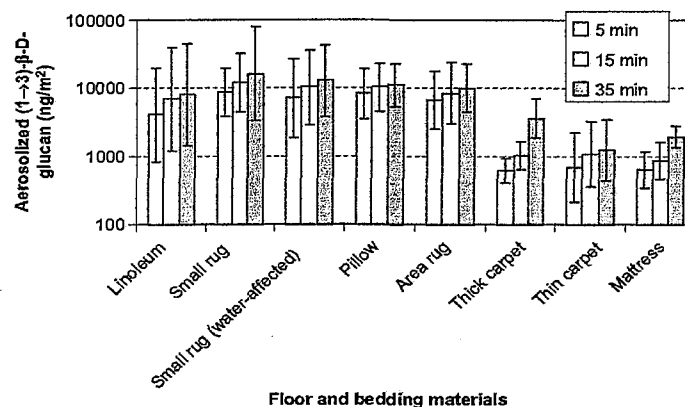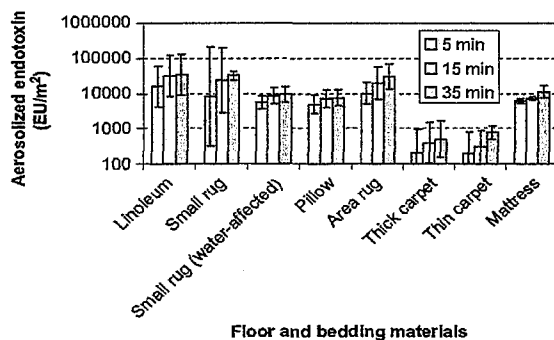

Fig. 4. Endotoxin aerosolized from contaminated materials collected in flood-affected homes of New Orleans. The histograms present geometric mean of three replicates and error bars indicate geometric standard deviations.

endotoxin collected with the vacuum cleaner did not show any significant correlation with the corresponding data obtained with the FSSST [for $(1\rightarrow3)$-β-D glucan: $r = 0.143$; $P = 0.736$; for endotoxin: $r = -0.405$; $P = 0.320$]. It should be noted that the FSSST data in Table 4 represent the samples generated by the first 5-min sampling to relate these to the vacuum samples collected over the same time interval.

3.7. Levels of dust mite allergens

The collected amounts of dust mite allergens (both Der f1 and Der p1) were below the detection limit in all FSSST samples. Following this finding, additional experiments were performed. Approximately 200 mg of dust mite allergen-containing dusts (supplied by Healthy Housing Solutions, Inc., Columbia, MD, USA) were spread on a 121 cm² area, and then samples were collected from the area using the FSSST. All nine samples analyzed from this additional experiment also failed to show any detectable levels of dust mite allergens. Of the dust samples collected by vacuum

Table 4

Comparison of aerosolized $(1\rightarrow3)$-β-D-glucan and endotoxin levels collected by FSSST (first 5-min samples) and a vacuum cleaner.

Materials	Aerosolized $(1\rightarrow3)$-β-D-glucan $(\times 10^3\,\text{ng/m}^2)$			Aerosolized endotoxin $(\times 10^3\,\text{EU/m}^2)$		
	FSSST (first 5 min); mean±SD $(n=3)$	Vacuum cleaner $(n=1)$	Ratio (vacuum cleaner: FSSST)	FSSST (first 5 min); mean±SD $(n=3)$	Vacuum cleaner $(n=1)$	Ratio (vacuum cleaner: FSSST $\times 10^3$)
Linoleum	8.72±11.37	27.47	3	15.26±10.21	1678.81	0.11
Small rug	10.48±6.28	2061.44	197	57.53±99.12	4032.62	0.07
Small rug (water-affected)	13.22±17.02	107.80	8	4.65±4.03	2333.09	0.50
Pillow	10.32±8.25	8.20	0.8	3.05±4.43	1535.57	0.50
Area rug	8.62±5.77	906.47	105	7.12±8.06	820.90	0.15
Thick carpet	0.59±0.33	161.43	273	0.39±0.44	6144.50	15.83
Thin carpet	1.09±1.24	344.24	316	0.31±0.23	5868.17	19.17
Mattress	0.71±0.34	2.04	3	6.09±1.13	2671.82	0.44

cleaners, detectable Der f1 was found only in the pillow (0.38 µg/g) and the small rug (0.4 µg/g); detectable Der p1 was found only in the small rug (1.3 µg/g).

4. Discussion

Results obtained with the FSSST for the levels of aerosolized culturable fungi were above detection limit in five materials, ranging up to $(2.59\pm3.53)\times10^5\,\text{CFU/m}^2$. Measured levels of total aerosolized fungi in all materials ranged from approximately 2.1×10^5 to 1.6×10^6 spores/m^2. Previously, FSSST had been used in a field study by Niemeier et al. (2006) to investigate aerosolization of culturable and total fungal spores in visibly mold-contaminated homes in Ohio. The quoted study reported approximately $10^5\,\text{CFU/m}^2$ of aerosolized culturable fungi and 10^6 spores/m^2 of aerosolized total fungal spores. While generally of the same order of magnitude, for several materials, the aerosolized concentration levels obtained in the present study were lower than those reported by Niemeier et al. (for both the culturable and total fungi). It should be noted that Niemeier et al. selected particularly moldy wall surfaces for the FSSST application, whereas we applied a random selection of sampling surfaces, irrespective of the signs of visible mold or water damage. The levels of culturable fungi aerosolized in our tests with the mattress (which was slightly moist at the time of collection) were comparable to those reported in Niemeier's study.

We found levels of aerosolized culturable fungi in many of the FSSST samples, consistently below the lower limit of detection. However, based on our observations of culturable fungi levels on surfaces by swabbing and extraction, as well as previously-measured aerosolization ratios for fungal spores [1–2% for *Aspergillus/Penicillium* as reported in Sivasubramani et al. (2004b)], detectable levels of aerosolized culturable fungi were expected in most samples. These expectations were unmet, potentially due to: (a) mud or sediment dust layers inhibiting germination, and/or (b) varying species of fungi present in different materials with disparate culturability in the selected culture media.

Culturable fungi were identified up to the genus level, and colonies of three fungal genera were identified: *Aspergillus*, *Penicillium*, and *Cladosporium*, along with non-sporulating colonies. We found that swabbing provided a similar concentration of viable fungi to that determined with the FSSST for the mattress, in contrast to the similar previous comparison made by Niemeier et al. (2006). Although swabbing is routinely used for environmental sampling, the levels of collected viable fungi using swabbing may not be as appropriate as FSSST, particularly for the porous mattress surfaces. While analyzing aerosolized total fungal spores, twelve fungal genera and classes were identified;

Aspergillus/Penicillium was the most prevalent in all samples, except for the mattress (Table 3). The concentration of *Stachybotrys*, however, was highest in the mattress, which was moist at the time of collection. Similar to Niemeier et al. who utilized the FSSST for enumerating total fungal spores in field samples, we also observed common occurrences of *Aspergillus/Penicillium*, *Cladosporium*, Ascospores, *Chaetomium*, and *Stachybotrys*. Additionally, we frequently found the presence of *Alternaria*, *Botrytis*, and Basidiospores. While comparing the occurrence of culturable fungi with Niemeier et al.'s study, we found significantly lower species variability. In addition to *Aspergillus*, *Penicillium*, *Cladosporium*, and non-sporulating colonies (identified in the present study), Niemeier et al. also recorded colonies of *Mucor*, *Paecilomyces*, *Pithomyces*, *Stachybotrys*, and *Epicoccum* in FSSST samples. Among different fungal genera that we isolated in the present study, *Cladosporium*, *Aspergillus*, *Penicillium*, and *Alternaria* have been strongly associated with allergic respiratory disease, especially asthma (Douwes et al., 2003). The relationship between increased risk of building-associated pulmonary disease and the presence of *Stachybotrys* in bulk surface samples has been reported by several investigators (Hodgson et al., 1998).

This investigation is the first one that utilized the FSSST with the field samples for quantifying the aerosolization of $(1\rightarrow3)$-β-D glucan and endotoxin. The average levels of aerosolized $(1\rightarrow3)$-β-D glucan and endotoxin in all materials were approximately 2.0×10^3–2.9×10^4 ng/m^2 and 7.0×10^2–9.3×10^4 EU/m^2, respectively. In a recent study by this group (Seo et al., 2008) the FSSST was used to characterize the aerosolization of $(1\rightarrow3)$-β-D glucan in the laboratory from ceiling tile and gypsum board samples artificially contaminated by *Aspergillus versicolor* and *Stachybotrys chartarum*. The reported concentrations of aerosolized glucan ranged from 2.0×10^0 to 1.6×10^4 ng per material sample (area of 60 cm^2), which corresponds to the range 3.3×10^2–2.7×10^6 ng/m^2. The elevated glucan concentration range observed by Seo et al. can be explained by the difference in the initial contamination levels of materials used in these two studies.

Vacuuming has traditionally been used for the collection of $(1\rightarrow3)$-β-D glucan and endotoxin in field studies. The levels of $(1\rightarrow3)$-β-D glucan obtained in this study, by vacuuming five flood-affected materials obtained in New Orleans homes (ranging from 2.04×10^3 to 2.061×10^6 µg/m^2), were generally higher than those determined from the vacuum-collected dust samples in 574 Cincinnati homes in a report recently published by Iossifova et al. (2007) (geometric mean = 18.4 µg/m^2). Similarly, the endotoxin levels in all materials collected by vacuuming $(8.21\times10^5$–6.144×10^6 EU/m$^2)$ were approximately 10^3–10^5 times higher than in dust samples collected from homes in New York $(3,892\,\text{EU/m}^2$; Perzanowski et al., 2006) and Cincinnati $(24\,\text{EU/m}^2$; Iossifova et al., 2007).

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The amounts of dust mite allergens in all FSSST samples collected from different materials were below the detection limit. We assumed that dust mites either rarely infested flood-affected materials or their allergens were altered in some way that precluded measurement. Interestingly, the materials, which aerosolized very low amounts of culturable fungi (including those <LOD), produced dust samples (collected by vacuuming) with detectable levels of dust mite allergens. This finding suggests that an antagonistic ecological relationship between dust mites and species of fungi present in these materials is possible. Since we could not detect aerosolizable dust mite allergens in most samples, their potentially inhibitory roles in fungal aerosolization and stimulatory roles in aerosolization of $(1\rightarrow3)$-β-D glucan have to be investigated.

Standardization of sampling time is important while characterizing aerosolized microorganisms and hazardous microbial substances using a new technique. Since the application of FSSST is not yet conventional, and no sampling protocol is available for prospective future field studies, we conducted a series of experiments here to compare the aerosolized biocontaminant levels collected during cumulative 5, 15, and 35 min with the FSSST. For the level of total aerosolized fungi and $(1\rightarrow3)$-β-D glucan, the results obtained at 15 min did not significantly differ from 5 to 35 min levels. For endotoxin, no differences were found between the results obtained at the three sampling times. These observations indicate that FSSST operation for 15 min would be sufficient to collect aerosolizable total fungi and $(1\rightarrow3)$-β-D glucan from the surfaces of flood-affected materials, and a shorter 5 min sampling period can be considered for collecting aerosolized endotoxin.

When comparing the aerosolized biocontaminant levels among different materials, we found wide inter- and intra-sample variations. The moist mattress had the maximum levels of culturable and total fungi; however, there must be other factors for the growth of culturable fungi besides moisture, such as availability of nutrients and growth-supporting materials. These two factors may also influence the variability of fungal flora on surfaces and interactions between various species. Unlike with the mattress, we found only a few culturable fungi aerosolized from the water-affected small rug, which was moist at the time of collection. Higher concentrations of aerosolized $(1\rightarrow3)$-β-D glucan were observed in small rugs and linoleum, and lower concentrations were found in the mattress. The latter is particularly interesting, since the highest levels of culturable and total fungi were aerosolized from the mattress. These contrasting observations indicate that the major sources of $(1\rightarrow3)$-β-D glucan could be different from viable and non-viable fungi. Fragmented fungal mycelium or fungal spore-bearing structures, plant material or other sources (unknown at the time) may have been among these sources. Different species of fungi may have different $(1\rightarrow3)$-β-D glucan content (Fogelmark and Rylander, 1997). As we found higher concentrations of *Stachybotrys* and *Chaetomium* in the mattress and, in contrast, *Alternaria* and *Cladosporium* in linoleum and small rugs, the data point to a difference in primary contaminant fungal species. Higher concentrations of aerosolized endotoxin were observed in small rugs and linoleum and lower concentrations were found in carpets, pillow, and mattress. The moist small rug did not show an elevated level of endotoxin, indicating that water activity may not be directly related to endotoxin levels. Large variations of $(1\rightarrow3)$-β-D glucan and endotoxin in different samples can be attributed to the different levels of fungal and bacterial growth, depending on moisture availability or sediment deposition. Large variations were also observed from the different positions of the same samples, which may be related to different levels of water damage or availability of organic nutrients in different portions of the materials. These

observations should be important for the future field studies in flood-affected and water-damaged homes. We found a significant positive correlation between the aerosolized $(1\rightarrow3)$-β-D glucan and endotoxin levels. This is consistent with several previous studies (Schram-Bijkerk et al., 2005; Douwes et al., 2006; Iossifova et al., 2007). As stated in the introduction, floodwater sediment with decomposing organic materials can support growth of microorganisms, including fungi and bacteria, equally. Thus, this positive significant correlation between the aerosolized $(1\rightarrow3)$-β-D glucan and endotoxin levels is feasible.

This study gives unique new size-selective data on the aerosolization of microbial components. We found that the size fractions of <1 μm and 1–1.8 μm have comparable levels of $(1\rightarrow3)$-β-D glucans and endotoxin to the one determined for >1.8 μm in most of the materials. This is an important finding because it suggests that smaller respirable particles may have levels of $(1\rightarrow3)$-β-D glucan and endotoxin as high as larger particles; this raises considerable concern regarding exposure to small-size biocontaminants, which can be aerosolized in flood-affected homes. These observations also indicate that smaller particle size fractions of microbial biocontaminants contribute significantly to the total inhalation exposures. Since submicron fungal fragments can be a major source of $(1\rightarrow3)$-β-D glucan, the above finding has strong implications in the respiratory health effects of fungi. The effect of muddy flood water damage on the microbial growth in our selected materials may be different than regular moisture damage in homes; however, we believe these findings have general significance and would be applicable for the release of $(1\rightarrow3)$-β-D glucan and endotoxin from moist surfaces.

Higher levels of $(1\rightarrow3)$-β-D glucan and endotoxin were obtained in most dust samples collected with the vacuum cleaner, as compared to the levels measured with the FSSST (exceeding 10^2 fold in some samples for $(1\rightarrow3)$-β-D glucan and ranging from about 10^2 to $>10^4$ fold for endotoxin). A contrasting result was observed only for the pillow, when glucan in dust was slightly lower than in the FSSST sample. The soft surface of the pillow may have blocked the vacuum cleaner nozzle and inhibited collection. The high magnitude of the differences and the lack of correlation between the FSSST and vacuum cleaner data suggest that a vacuum cleaner can overestimate inhalation exposure risks of biocontaminants. Since a vacuum cleaner collects both the aerosolizable biocontaminants and those adhering to the surface, these findings are explainable.

The present study allowed for the characterization of aerosolizable fungi, $(1\rightarrow3)$-β-D glucan, and endotoxin per square meter area (overall and in different particle size fractions) in the worst case situation, when the materials were collected from homes heavily affected by a major water damage. One of the challenges of this study was associated with the large coefficient of variations in biocontaminants' levels observed in different materials. This suggests that more flood-affected materials must be studied, and more repeat samples should be collected from different positions to achieve an enhanced understanding of the collective aerosolization strengths of these biocontaminants in flood-affected homes. The results of the study, pertaining to the aerosolization of fungi, $(1\rightarrow3)$-β-D-glucan, and endotoxin, as well as the size-selective data for the above factors will supplement the previous findings of Chew et al. (2006) and Rao et al. (2007), who also have investigated airborne concentration levels of these biocontaminants in flood-affected homes of New Orleans. Chew et al. (2006) found culturable fungi levels ranged from 22,000 to 515,000 colony-forming units (CFUs)/m^3, fungal spore counts were between 82,000 and 630,000 spores/m^3, and endotoxin levels ranged from 17 to 139 endotoxin units (EU)/m^3. Rao et al. (2007) reported rather high concentrations of airborne fungi, endotoxin, and $(1\rightarrow3, 1\rightarrow6)$-$\beta$-D-glucans in New Orleans homes,

as well as in outdoor locations after Hurricanes Katrina and Rita. The air sampling time, however, was very short in the quoted studies: 1–20 min and 36–144 min, respectively. It is not known how adequately the biocontaminant exposure levels are represented by these short periods of air sampling. Our approach was to estimate the worst case scenario corresponding to particularly intense aerosolization of biocontaminants. By using the FSSST, this can be achieved with short sampling periods of ≤20 min. Moreover, the quantitative characterization of source strength of different flood-affected materials for aerosolizing these biocontaminants was beyond the scope of studies conducted by Chew et al. and Rao et al. Overall, the data reported in these two papers, which are considerably complementary to the findings of this study, provide a better understanding of the exposure and inhalation risks to fungi, $(1\rightarrow3)$-β-D-glucan, and endotoxin in flood-affected homes in New Orleans.

This study is characterized by some limitations that can be addressed in future investigations. First, a limited number of replications resulted in a rather wide intra-sample variation in the biocontaminant levels. Second, while the choice of materials for testing accounted—among other factors—for their typical use in a household, we acknowledge that some of the tested materials (especially highly-contaminated) may be discarded by the residents during restoration and thus will not anymore represent a source of long-term exposure for the occupants. A follow-up comparative study of restored homes would be appropriate to better address the relevance issue. Furthermore, stratification of biocontaminated materials based on their composition would be useful in future studies because synthetic and organic matters may have different abilities of supporting microbial growth under the same environmental conditions.

The present investigation has several practical implications for further field studies involving techniques for assessing the aerosolization of fungi and other flood/moisture-related indoor biocontaminants from surfaces. It has been proven that the FSSST can efficiently release aerosolizable fungi, $(1\rightarrow3)$-β-D glucan, and endotoxin from contaminated surfaces over a relatively short sampling time. A period of 5 min is recommended for field sampling of endotoxins, while 15 min seems to be appropriate for fungi and $(1\rightarrow3)$-β-D glucan. Although vacuum cleaners are routinely used for indoor testing, our results indicate that they can overestimate the inhalation exposure risks of aerosolizable surface biocontaminants; parallel investigations of surface biocontaminants, using the FSSST, are highly desirable. The aerosolization rate and size-selective characteristics of fungi, $(1\rightarrow3)$-β-D glucan, and endotoxin aerosolized from different materials, which have been quantified in this study, can be used in further exposure assessment research, particularly for estimating exposures to sub-micron aerosol fractions of these biocontaminants. Our results imply that the monitoring of sub-micron fractions of different microbial biocontaminants and their potential toxicity, followed by a contaminated building renovation, is required to ensure satisfactory control of microbial air pollution in flood-affected buildings. In addition, these results will be useful for developing guidance on respiratory protection against fungal spores during mold remediation. Overall, the information generated in this study is important with respect to restoration and rejuvenation of the flood-affected areas of New Orleans. According to the US Department of HUD, the estimated number of homes in Orleans parish that received 2 ft or more of floodwater after Hurricane Katrina was 103,513 out of 215,101 (HUD, 2007). As with a majority of these homes, those selected for this investigation remained untreated, with mold, dust and water damage visible on the walls and floors. Complete remediation of the flood-affected homes in New Orleans will likely take years, and many policy and logistical decisions in this context have yet to be made.

Beyond dealing with consequences of Katrina and Rita, we believe that the lessons learned in this study will be of significant help for assessing burden of microbial contaminants during similar disasters in other regions of the world including major coastal floods from tsunamis, hurricanes, and tornados.

5. Conclusions

In conclusion, significantly higher levels of aerosolized $(1\rightarrow3)$-β-D glucan and endotoxin were observed in the flood-affected materials collected in New Orleans, as compared to other studies conducted in urban homes. At the same time, the levels of culturable and total fungi found in these materials were slightly lower than those previously reported for moldy buildings. A significant positive correlation existed between the aerosolized $(1\rightarrow3)$-β-D glucan and endotoxin levels. Wide variations of all three biocontaminant levels were observed in different materials. Smaller aerosolized particles ($<1.8\,\mu m$) were found to have the $(1\rightarrow3)$-β-D glucan and endotoxin levels comparable to larger ($>1.8\,\mu m$) particles. Since finer particles can be inhaled deeper into airways, this finding raises additional exposure concerns. Short sampling periods of 5 min for endotoxin and 15 min for fungi and $(1\rightarrow3)$-β-D glucan were found to be sufficient for detecting most aerosolizable biocontaminants with the FSSST.

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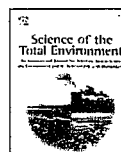
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Exposure matrices of endotoxin, (1→3)-β-D-glucan, fungi, and dust mite allergens in flood-affected homes of New Orleans

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ABSTRACT

This study examined: (i) biocontaminant levels in flooded homes of New Orleans two years after the flooding; (ii) seasonal changes in biocontaminant levels, and (iii) correlations between biocontaminant levels obtained by different environmental monitoring methods. Endotoxin, (1→3)-β-D-glucan, fungal spores, and dust mite allergens were measured in 35 homes during summer and winter. A combination of dust sampling, aerosolization-based microbial source assessment, and long-term inhalable bioaerosol sampling aided in understanding exposure matrices. On average, endotoxin found in the aerosolized fraction accounted for <2% of that measured in the floor dust, suggesting that vacuuming could overestimate inhalation exposures. In contrast, the (1→3)-β-D-glucan levels in the floor dust and aerosolized fractions were mostly comparable, and 25% of the homes showed aerosolizable levels even higher than the dust-borne levels. The seasonal patterns for endotoxin in dust and the aerosolizable fraction were different from those found for (1→3)-β-D-glucan, reflecting the temperature and humidity effects on bacterial and fungal contamination. While the concentration of airborne endotoxin followed the same seasonal trend as endotoxin aerosolized from surfaces, no significant seasonal difference was identified for the concentrations of airborne (1→3)-β-D-glucan and fungal spores. This was attributed to the difference in the particle size; smaller endotoxin-containing particles can remain airborne for longer time than larger fungal spores or (1→3)-β-D-glucan-containing particles. It is also possible that fungal aerosolization in home environments did not reach its full potential. Detectable dust mite allergens were found only in dust samples, and more commonly in occupied homes. Levels of endotoxin, (1→3)-β-D-glucan, and fungi in air had decreased during the two-year period following the flooding as compared to immediate measurements; however, the dust-borne endotoxin and (1→3)-β-D-glucan levels remained elevated. No conclusive correlations were found between the three environmental monitoring methods. The findings support the use of multiple methods when assessing exposure to microbial contaminants.

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1. Introduction

Human inhalation exposure to microbial contaminants and allergens in indoor environments can be determined by measuring the concentrations of these pollutants using various air sampling methods and collecting dusts with a conventional vacuuming method (HUD, 2004). In addition, dust aerosolizable from surfaces can be sampled using an unconventional microbial source testing method developed and validated by our group (Grinshpun et al., 2002; Sivasubramani et al., 2004a,b). To the authors' knowledge, there is a lack of scientific data on exposure matrices for different microbial contaminants sampled by different environmental monitoring methods. We have used 'exposure matrix' here referring to simultaneous assessment of exposure to various microbial contaminants in air, dust, and from surfaces within which the real inhalation exposure originates and develops. The relationship between various bioaerosol exposure assessment methods is worthwhile to investigate. This would allow building an ideal exposure assessment strategy that can be adopted during a natural disaster, e.g., a major flood that can create profuse inhalation exposure risks to microbial contaminants, implicating significant public health concerns.

Hurricanes Katrina and Rita caused unprecedented flooding and disasters in New Orleans in August and September of 2005. Moisture damage in flood-affected homes provided ample ideal niches for the growth of fungi and bacteria, resulting in increased risk of exposure to some biocontaminants derived from these microorganisms. Due to

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the moisture damage and warm, damp conditions inside of the flood-affected homes, visible mold growth was detected in approximately 44% of homes (Riggs et al., 2008). Moisture damage could also promote bacterial growth in flood-affected materials. Fabian et al. (2000) found that total indoor bacterial counts exceeded outdoor counts by two orders of magnitude or more in water-damaged homes, whereas all samples from non-water damaged homes had lower bacterial counts indoors than outdoors. Flooded homes could be a significant source of airborne endotoxin (Rao et al., 2007).

Arlian et al. (1992) reported high average dust mite densities in New Orleans homes prior to Hurricanes Katrina and Rita. The elevated humidity, followed by major floods caused by these hurricanes, is expected to optimize the growth conditions for dust mites because previous researchers found significant increases in dust mite population in laboratory cultures at higher relative humidity levels (Voorhorst et al., 1969).

Airborne fungi, bacteria, their byproducts, and dust mite allergens are potential respiratory health hazards (Thorn, 2001; Douwes et al., 2003; Rylander et al., 1998; Rylander, 1999; IOM, 2000). Aerosolized particles can be inhaled by returning residents and renovation workers in flood-affected homes. Several studies have reported high exposure levels of the above listed contaminants (except dust mite allergens) in New Orleans homes. Chew et al. (2006) investigated three homes in three time periods: before, during and after renovation. It was observed that concentrations of airborne culturable fungi before renovation ranged from 22×10^3 to 515×10^3 colony-forming units (CFUs)/m^3, airborne total fungal spore levels ranged from 82×10^3 to 630×10^3 spores/m^3, and endotoxin levels in air ranged from 17 to 139 endotoxin units (EU)/m^3. In a 20-home study, Rao et al. (2007) found the following geometric mean concentrations: 280×10^3 spores/m^3 for airborne fungi, 1.6 μg/m^3 for $(1 \rightarrow 3, 1 \rightarrow 6)$-β-D-glucans, and 23.3 EU/m^3 for endotoxin. Schwab et al. (2007) collected air samples in six homes and found even higher airborne fungal spore concentrations (up to 735×10^3 spores/m^3). It is to be noted that the air sampling time periods in the above-quoted three studies were very short: 1 to 20 min (Chew et al.); 36 to 144 min (Rao et al.); and 10 to 17 min (Schwab et al.). Solomon et al. (2006), who conducted air sampling during 6 h and 24 h in 8 homes, found indoor concentrations of airborne fungal spores ranging from 11×10^3 to 645×10^3 spores/m^3. The investigators reported that the 6-h time-averaged concentration measured from 9 AM to 3 PM differed from the 24-h time-averaged one at each site, although the difference was not consistent in either magnitude or direction and was not statistically significant. Solomon et al. measured airborne endotoxin levels only inside two homes; they ranged from 4.5 to 7.3 EU/m^3. There were several noteworthy limitations in the referred studies, such as: (a) the small sample size; (b) short air sampling period in most cases; (c) deployment of samplers with d_{50} cut-off size above the size of dry spores of common fungal species, including some Aspergillus and Penicillium (e.g., Solomon et al. used Burkard sampler with $d_{50} = 2.52$ μm; Schwab et al. used VersaTrap samplers with $d_{50} = 2.3–2.7$ μm); (d) lack of replicate measurements [except Chew et al.'s (2006) study]. Thus, the investigations performed up-to-date have limited applicability in assessing exposure levels of the returning occupants or renovation workers. The present study was initiated to address the above-listed limitations.

The main purpose was to examine the exposure matrices of endotoxin, $(1 \rightarrow 3)$-β-D-glucan, fungi, and dust mite allergen (Der f 1, and Der p 1) levels in 35 unoccupied or partially occupied flood-affected homes of New Orleans from April to August, 2007 and from November 2007 to February, 2008. These exposure matrices were examined through long-term inhalable air sampling, sampling of contaminants aerosolizable from surfaces, and conventional dust sampling methods. In parallel to these measurements, environmental temperature, relative humidity, surface moisture, conditions of floor materials, and applications of bleach or other chemicals during the

two seasons were recorded. The following research questions were addressed in the study:

(i) What are the exposure levels of a few specific health-hazardous biocontaminants in the flood-affected homes two years after the flooding relative to the levels recorded within 2–6 months following the flooding?
(ii) Was there any seasonal change in these biocontaminant exposure levels between summer and winter in the flood-affected homes?
(iii) Do the exposure levels of the selected biocontaminants assessed by different environmental monitoring methods correlate?

2. Materials and methods

2.1. Selection of flood-affected homes in New Orleans area

We selected 35 moisture-problem homes from a pool of approximately 200 households affected by the flooding during Hurricanes Katrina and Rita, identified with help from local community organizations and agencies. The initial home inclusion criteria were: (1) at least 3 ft in height of flooding in the homes, which represented homes with heavy mold contamination, and (2) residents were dwelling in the home overnight, which allowed using exposure data in other prospective epidemiological studies involving the affected residents, as well as repeat exposure analysis of the same home. We anticipated that most floors in the selected homes would represent untreated hardwood or carpet floor materials. In a few instances, we found that flooded hardwood or carpet floor materials had been removed, and only concrete slabs remained. Moreover, we found that floors were sometimes treated with bleach and other chemicals. We expected that residents would mostly stay overnight in their homes; however, in most cases, they were staying during the day time (primarily doing renovation work), but not necessarily overnight. The characteristics of the selected 35 homes are summarized in Table 1. As shown in the table, most of the homes had hardwood floors, showed signs of water damage and visible mold, and remained partially occupied or unoccupied during the sample collection. Among chemicals used for remediation in homes, bleach was the most common. All 35 homes were tested in the summer campaign, but only 31 were available for testing in the winter

Table 1
Characteristics of 35 flood-affected homes investigated in the present study.

Home characteristics	Categories	Number of homes (n)	Percentage (%)
Floor materials	Carpet	4	11
	Hardwood	20	57
	Concrete slab	6	17
	Tile	3	9
	Linoleum	1	3
	Plywood	1	3
Signs of water damage	Yes	31	89
	No	4	11
Signs of visible mold	Yes	34	97
	No	1	3
Occupation status	Unoccupied	27	77
	Occupied	8	23
Bleach and other chemicals treatment status	Treated	19	54
	Untreated	16	46
Chemicals treatment type	Microban	2	11
	Boracare	3	16
	Jomax	1	5
	Sterifab and Virex	1	5
	Zinase	1	5
	Bleach	8	42
	Unknown chemicals	3	16

campaign because the home owners were not willing to continue their participation in the study.

2.2. Survey and sampling methods

2.2.1. Walkthrough

A home walkthrough checklist was developed utilizing existing checklists (HUD, 2004; Cho et al., 2006). It included the size of the home, presence of pets, moisture problems and/or visible mold, as well as the measured temperature and humidity. In homes with visible mold and moisture problems, the moisture content of the test surface was measured with a Protimeter (Protimeter Surveymaster, GE Sensing & Inspection Technologies, Billerica, MA). The air temperature and relative humidity were determined with a thermo-hygrometer pen (Fisher Scientific, Pittsburgh, PA).

2.2.2. Sampling strategy

Conventional dust and air sampling methods, vacuuming and filtration-based inhalable aerosol sampling, were employed. We also utilized a source assessment method aiming at determining the aerosolization potential of specific biocontaminants from surfaces. This was done using a novel microbial source tester validated and used in our earlier studies (Grinshpun et al., 2002; Górny et al., 2002; Sivasubramani et al., 2004a,b; Niemeier et al., 2006; Seo et al., 2008; Adhikari et al., 2009), in which it was referred to as the Fungal Spore Source Strength Tester or FSSST. In each home, a bedroom with a floor area of 50–300 ft^2 was selected for the field sampling. Our original plan was to collect five samples from each home: two samples by vacuuming the floor and the mattress, two samples with the source tester applied to the floor and the mattress, and one air sample. However, we found that the flood-affected mattresses were already discarded in most of the homes. In homes where no mattress was available, only three samples were collected. In most cases, the residents were not present during sample collection; however, if present, they continued their normal activities during measurements.

All the collected samples were immediately brought to the laboratory, extracted following standard methods, and divided into aliquots and preserved at −20 °C. The samples were then analyzed for endotoxin, (1→3)-β-D-glucan, total fungal spores, and dust mite allergens, as described below.

The measurements were conducted in two campaigns — "summer" (late spring through summer) and "winter" (late fall through winter). As the flood-affected homes were mostly devoid of electricity, we assumed that seasonal changes of temperature and humidity could have significantly affected the microbial growth in these environments.

2.2.3. Sample collection

Dust samples were collected from the floor into a small filter bag, by vacuuming with a Filter Queen Majestic® vacuum cleaner (Health-Mor, HMI Industries Inc., Seven Hills, OH) for 5 min from 1 m^2 of the floor area. The HUD Standard Dust Sampling Protocol (HUD, 2004) was followed. For mattresses, 2-m^2 areas were vacuumed for 5 min. Collected dust samples were sieved (355 μm sieve), and the resulting fine dust was divided into sub-samples and stored at −20 °C, until the analysis.

When assessing the source aerosolization potential, the aerosolization chamber of the FSSST was tightly held against the floor or mattress surface, covering an area of 0.012 m^2. A push vacuum pump produced an airflow that passed through a HEPA filter (PALL Gelman Laboratory, Ann Arbor, MI). The incoming air flow directed through the 112-orifice stage at the bottom of the device created air jets towards the floor or mattress surface, and the particles containing above-mentioned microbiological contaminants were aerosolized by these air jets and collected into a BioSampler (SKC, Inc., Eighty Four, PA). The sampler was located at the outlet of the aerosolization chamber and operated by a pull vacuum pump at a flow rate of 12.5 L/min. The collection vessel in

the BioSampler was filled with 20 mL suspension of pyrogen free sterile water mixed with 0.05% Tween 80. The flow rate balance was adjusted during sampling so that the incoming air flow was always slightly (by 0.5–1.0 L/min) lower than the sampling air flow. This prevented contaminating the indoor air by the FSSST operation. The device was thoroughly cleaned between the tests with 70% ethyl alcohol and air dried in a biosafety hood; a separate sterile BioSampler was used for each sample collection. After 15 min sampling (this duration was selected following our laboratory study; see Adhikari et al., 2009), the collection fluid of each BioSampler was divided into sub-samples and stored at −20 °C until analyzed. The results achieved by the aggressive sampling with the FSSST represent the aerosolization potential of microbial sources, which allows predicting maximum aerosol concentration for each microbial contaminant in each flood-affected home.

Air samples were collected in the middle of the bedroom, at a height of 1.1 m, using a Button Inhalable Aerosol Sampler (SKC Inc., Eighty Four, PA) equipped with 25-mm polycarbonate filters (pore size = 2 μm). The Button Sampler was operated at a flow rate of 4 L/min for approximately 24 h. The flow rates of all pumps were calibrated before and after each sampling. The Button Sampler was thoroughly cleaned and sterilized by autoclaving between the measurements. After the sampling, the sample filters were stored at −20 °C, until the extraction.

2.3. Analysis of samples collected by vacuuming, microbial source tester, and air sampling

2.3.1. Endotoxin and (1→3)-β-D-glucan

For the analysis of endotoxin and (1→3)-β-D-glucan in dust samples, 25 mg of fine dust was used for extraction of each. Endotoxin was extracted in 1.0 mL of pyrogen free sterile water for 1 h by sonication, whereas (1→3)-β-D-glucan was extracted in 1.0 mL of 0.6 M NaOH solution by vigorously shaking. Both types of extracts were then centrifuged at 7000 rpm (5204×g) for 1 min. Supernatants were collected for the analysis. Button Sampler filters were previously extracted into 5 mL of pyrogen-free water containing 0.05% Tween 80 by vortexing and sonication and stored at −20 °C for several days until analysis. FSSST's Biosampler suspensions were directly stored at −20 °C. Immediately before analysis, these extracts and previously stored suspensions from FSSST's BioSampler (0.5 mL aliquots) were again either sonicated for endotoxin or vigorously shaken for (1→3)-β-D-glucan as described previously.

For endotoxin measurement, the supernatants from dust samples and sonicated extracts from the Button Sampler and Biosampler were analyzed with the endotoxin-specific kinetic chromogenic Limulus Amebocyte Lysate (LAL) assay (Pyrochrome, Associates of Cape Cod, East Falmouth, MA). Endotoxin concentrations in liquid extracts were converted into EU/m^2 for dust and FSSST samples and in EU/m^3 for air samples derived from the weight of total sieved dust collected and dust used for extraction, area sampled, air flow rates, and sampling duration. The lower limit of detection (LLOD) for endotoxin in suspension was 0.05 EU/mL.

For (1→3)-β-D-glucan measurement, the supernatants from dust samples and sonicated extracts from the Button Sampler and Biosampler were analyzed with the (1→3)-β-D-glucan-specific kinetic chromogenic LAL assay (Glucatell, Associates of Cape Cod), as previously described by Lee et al. (2006) and Iossifova et al. (2007). The (1→3)-β-D-glucan concentration values were presented in μg/m^2 for the dust and FSSST samples and in μg/m^3 for air samples derived similarly as described above for endotoxin. The LLOD of (1→3)-β-D-glucan in suspension was 2.53 pg/mL.

2.3.2. Microscopic analysis of total fungal spores

Total fungal spore enumeration was conducted in air and FSSST samples collected from 10 randomly selected flood-affected homes. Total fungal spores in dust samples were not analyzed, because dust particles obstructed microscopic visualization. The filters from Button

Samplers were extracted into 5 mL of pyrogen-free water containing 0.05% Tween 80, and suspensions from FSSST's BioSampler were directly used for analysis. One milliliter aliquot of the Button Sampler filter extract (5 mL) and the 5 mL aliquot of the FSSST's BioSampler suspension (20 mL) were filtered through a 13-mm mixed cellulose esterase filter. Each filter was placed onto a slide and allowed to completely dry in a clean biosafety hood. The dry filter was made clear by treating with acetone vapor, following our previously developed protocols (Adhikari et al., 2003, 2004). Fungal spores were identified and counted in 40 microscopic fields (7.4% area of the filter) using a bright light microscope (Labophot 2, Nikon Corp., Japan) at a magnification of 400× (in addition, a higher magnification of 1000× was sometimes used for confirming the identification of smaller spores). Fungal spores were identified morphologically to genus/group level. Results were expressed in spores/m^2 for FSSST samples and in spores/m^3 for air samples. The LLOD of the fungal spore enumeration (derived from the limit of one spore per 40 microscopic fields and calculated accounting for the sampled area, air flow rate, and sampling time) was 5587 spores/m^2 for FSSST samples and 6 spores/m^3 for air samples.

2.3.3. Dust mite allergens

For dust mite allergen extraction, 100 mg of fine dust was extracted in 2 mL of phosphate-buffered saline with 0.05% Tween 20 and was shaken on a platform shaker for 1 h at 30 °C. These extracts were serially diluted in phosphate-buffered saline with 1% bovine serum albumin and 0.05% Tween 20 (BSA-PBS-T) solution at pH 7.4. Button Sampler filters were previously extracted into 5 mL of pyrogen-free water containing 0.05% Tween 80 by vortexing and sonication and stored at −20 °C. FSSST's Biosampler suspensions were directly stored at −20 °C. For these air and FSSST samples, undiluted or concentrated (using Amicon columns) filter extracts and suspensions were treated with BSA solution because Tween 80 had been already used for the extraction. In accordance to Luczynska et al. (1989), two-site monoclonal antibody (MAB) sandwich ELISAs (Indoor Biotechnologies, Inc., Charlottesville, VA) were used to analyze for Der p 1 and Der f 1. The analytical LLOD of the ELISA was 1.25 ng of allergen per mL of buffer. The sampling LLOD for dust samples was 0.025 ng/mg of dust collected (= 0.025 μg/g). Concentrating the FSSST extracts using Amicon columns allowed us to achieve a LLOD of 1.4–2.2 ng per air sample collected; the actual protein concentration varied by FSSST sample.

2.4. Statistical analysis

Prior to performing statistical analysis, the data were tested for distributions of the variables. The results of replicate measurements were averaged, and the standard deviations (SD) were calculated. Normality of the data distributions was tested by quantile–quantile or Q–Q plots. Pearson correlation (if normal distribution of data was achieved) or non-parametric Spearman's correlation coefficients (if normal distribution of data was not achieved) were calculated to characterize the association between the levels of endotoxin, (1 → 3)-β-D-glucan, fungal spores, dust mite allergens, and three measured environmental variables: temperature, relative humidity, and surface moisture. Statistical significance in the differences in seasonal variations of endotoxin, (1 → 3)-β-D-glucan, fungal spores, and dust mite allergens were calculated by paired t-test. The level of statistical significance was considered at p-values below 0.05. All statistical tests were performed using the SPSS 11.0 for Windows (SPSS Inc., Chicago, IL) software.

3. Results and discussion

3.1. Exposure matrices of endotoxin

Fig. 1 presents the median values and quartiles for endotoxin concentration in dust, its aerosolizable concentration (from FSSST), and
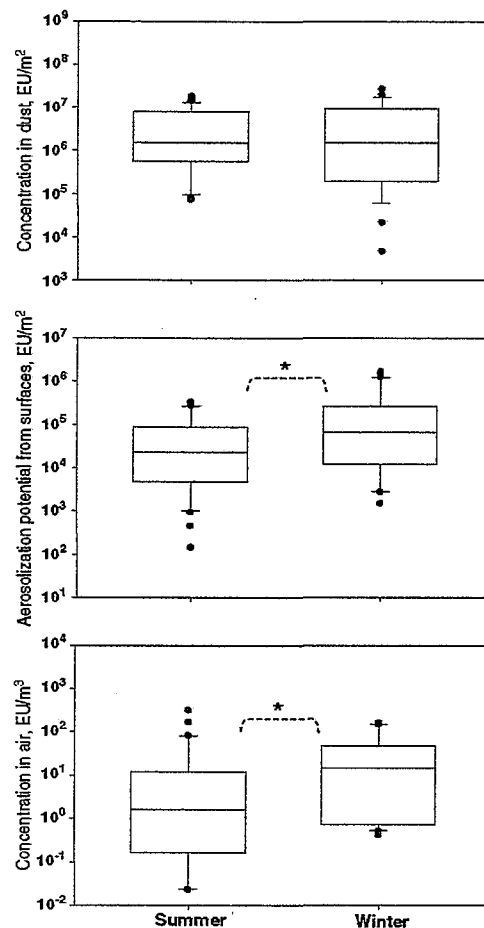

Fig. 1. Exposure matrices of endotoxin in flood-affected homes (n=35 in summer and 31 in winter). The lower and upper boundaries of the box specify the 25th and 75th percentiles, respectively. The line within the box indicates the median and the whiskers above and below the box indicate the 95th and 5th percentiles, respectively. * indicates statistically significant difference (paired t-test: p<0.05).

indoor air concentration. In addition, the following mean values and standard deviations were determined for the tested homes: (4.30 ± 5.22) × 10^6 EU/m^2 (dust), (6.87 ± 9.63) × 10^4 EU/m^2 (aerosolization potential) and 22.85 ± 60.95 EU/m^3 (indoor air) in summer, and (4.15 ± 5.77) × 10^6 EU/m^2 (dust), (2.78 ± 4.73) × 10^5 EU/m^2 (aerosolization potential) and 36.91 ± 50.94 EU/m^3 (indoor air) in winter.

No statistically significant difference in endotoxin levels measured in the two seasons was observed for dust samples (p>0.05). The total surface concentrations of the sieved fine dust collected by vacuuming during the two seasons were approximately the same (mean ± SD): 4.26 ± 2.18 g/m^2 (summer) and 4.02 ± 2.39 g/m^2 (winter). In contrast, statistically significant increases from summer to winter were observed for aerosolizable endotoxin (analyzed from the FSSST samples) and for the airborne endotoxin (analyzed from air samples). The aerosolization of endotoxin, as concluded from the FSSST results, was significantly higher in winter than in summer (p<0.05; paired t-test). This trend was observed in 71% of the homes and can be

attributed to the seasonal differences in air temperature and humidity; e.g., less humid air in winter (see Section 3.5) is likely to be associated with higher arosolization rates. Lower humidity might have facilitated desiccation of endotoxin-containing particles on surfaces, thus enhancing aerosolization. The concentration of airborne endotoxin followed the trend obtained for its aerosolization potential. As a component of the bacterial cell membrane, endotoxin is expected to be present primarily in the fine particle size fraction, including considerable amount in the submicrometer range. Recently published data on the endotoxin analysis of materials collected in flood-affected New Orleans homes (Adhikari et al., 2009) support the above expectation. Being very small, the endotoxin-containing particles, once aerosolized, are likely to remain airborne for a long time.

Additionally, the endotoxin aerosolization ratio was determined as its concentration in air divided by the concentration in vacuumed dust. This aerosolization ratio was higher in winter than in summer in 76% homes. This finding is consistent with the aerosolization potential data obtained using the FSSST.

Overall, we found that endotoxin levels in the vacuumed dust samples were mostly two orders of magnitudes higher than in the FSSST samples, indicating that only a small fraction of endotoxin (on average, <2%) was aerosolizable from the floor dust. This suggests that vacuuming could considerably overestimate the inhalation exposure risks associated with endotoxin released from the floors of the flood-affected homes. Similar findings were obtained in our recently published laboratory study (Adhikari et al., 2009), where the dust-borne endotoxin collected from flood-affected materials was 10^2- to 10^3-fold higher compared to its aerosolizable level (determined from the FSSST samples). It is acknowledged that vacuumed floor dust samples have a limited utilization for assessing the seasonal changes in exposure because contaminant levels in dust are less influenced by short-term variability in indoor activities than the corresponding levels in the air.

The dust-borne endotoxin levels obtained in the present study are approximately 10^2 to 10^4 times higher than those found in dust samples collected from homes in New York (3892 EU/m^2; Perzanowski et al., 2006) and Cincinnati (24 EU/m^2; Iossifova et al., 2007). This indicates that major flooding, such as the one which occurred in New Orleans, produces sustainable elevation of endotoxin in dust in flood-affected homes that remains high (as measured from dust samples), even two years after the flood. At the same time, the concentration of endotoxin measured in indoor air was only moderately elevated. Other investigators, who conducted indoor air monitoring in a more immediate aftermath of Hurricane Katrina, reported higher airborne endotoxin levels in flood-affected homes: a range of 17–139 EU/m^3 (Chew et al., 2006) and a geometric mean of 22.3 EU/m^3 (Rao et al., 2007). Recent measurements in non-moldy Cincinnati homes revealed a geometric mean of 8.7 EU/m^3 for airborne concentration of endotoxin (Reponen et al., 2009). For comparison, the geometric mean of airborne endotoxin obtained by integrating the summer and winter campaigns in our study was 3.03 EU/m^3 with a geometric standard deviation of 14.24 EU/m^3. This suggests that the airborne endotoxin levels in the flood-affected homes had decreased in two years after the floods, down to typical levels measured in non-damaged homes.

Although mattresses were not available in most homes, we had three pairs of samples (two from the summer and one from the winter campaign) that allowed comparing the endotoxin levels from the floor samples to those detected in mattresses. A proper statistical comparison is not possible with this limited amount of data. However, based on the mean values calculated from the vacuumed dust sample data, the endotoxin level on the floors was found to be approximately 5-fold higher than in mattresses: $(8.39 \pm 5.13) \times 10^5$ EU/m^2 versus $(1.70 \pm 0.64) \times 10^5$ EU/m^2. The FSSST samples demonstrated less pronounced difference: $(1.09 \pm 1.85) \times 10^5$ EU/m^2 (floors) versus $(0.68 \pm 1.14) \times 10^5$ EU/m^2 (mattresses).

We also compared the levels of endotoxin in chemically treated homes in summer versus winter using paired t-test and did not find any statistically significant difference of endotoxin in dust ($n=11$), FSSST ($n=15$), and air samples ($n=9$).

3.2. Exposure matrices of $(1 \rightarrow 3)$-β-D-glucan

The measured $(1 \rightarrow 3)$-β-D-glucan levels in dust, FSSST, and air samples are presented in a box plot format in Fig. 2 where data quartiles and medians are presented. In addition, for the summer dataset, the following mean values and standard deviations were calculated for the dust, FSSST, and air samples: $(15.50 \pm 28.89) \times 10^3$ µg/m^2, $(39.20 \pm 110.96) \times 10^3$ µg/m^2, and 15.92 ± 27.04 ng/m^3, respectively. The corresponding values in winter were lower: $(0.22 \pm 0.20) \times 10^3$ µg/m^2, $(0.17 \pm 0.87) \times 10^3$ µg/m^2, and 3.14 ± 2.05 ng/m^3.

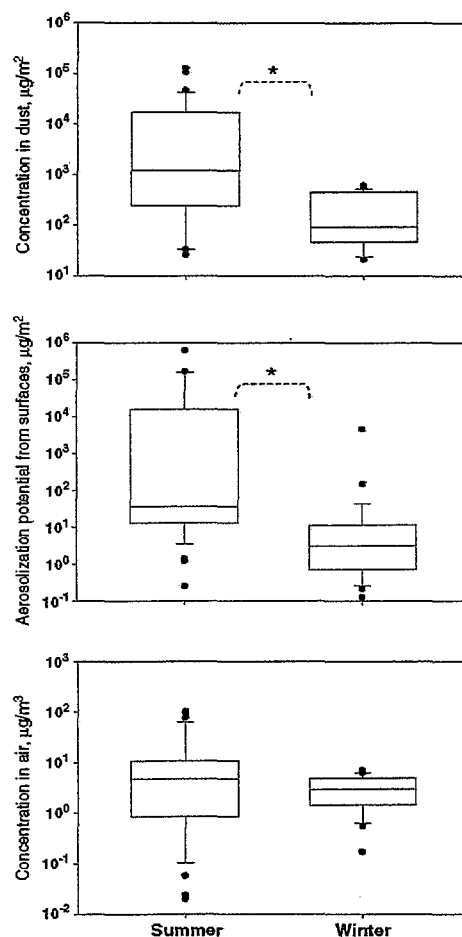Fig. 2. Exposure matrices of $(1 \rightarrow 3)$-β-D-glucan in flood-affected homes ($n=35$ in summer and 31 in winter). The lower and upper boundaries of the box specify the 25th and 75th percentiles, respectively. The line within the box indicates the median and the whiskers above and below the box indicate the 95th and 5th percentiles, respectively. * indicates statistically significant difference (paired t-test; $p<0.05$).

The $(1\rightarrow3)$-β-D-glucan measured on floor surfaces were compared to those obtained in mattresses in three pairs. No consistent differences were observed with respect to the $(1\rightarrow3)$-β-D-glucan concentration in either vacuumed dust or FSSST samples. This is likely because too few samples were available.

In contrast to endotoxin, the concentration of $(1\rightarrow3)$-β-D-glucan in dust and its maximum plausible aerosolizable fraction were lower in winter than in summer, perhaps reflecting inhibited fungal growth at lower temperature and humidity levels. Lower availability of moisture in the winter season could have inhibited the fungal growth on surfaces, which translated to lower levels of $(1\rightarrow3)$-β-D-glucan found in dust and consequently in the aerosolizable fraction. Unlike the dust samples and aerosolizable samples, the air samples did not reveal statistically significant seasonal difference with respect to $(1\rightarrow3)$-β-D-glucan. As a polyglucose molecule comprises a large amount of cell wall of most fungal spores, the presence of $(1\rightarrow3)$-β-D-glucan is linked to fungal spores or their fragments. The aerosolization rate of $(1\rightarrow3)$-β-D-glucan (by mass) is usually higher for larger particles (Adhikari et al., 2009). According to Seo et al. (2009), the concentration of $(1\rightarrow3)$-β-D-glucan in supermicrometer airborne fungal spores may be ~10^1- to 10^2-fold greater than that in submicrometer fragments. The aerodynamic sizes of single fungal spores and spore aggregates present in indoor air are usually above 2 μm and may even exceed 10 μm. These relatively large particles are characterized by significant gravitational settling velocities, which make most of them settle in homes within hours after aerosolization. This may explain why the concentration of $(1\rightarrow3)$-β-D-glucan in air did not follow the trends observed for the dust and aerosolizable samples. In addition, the aerosolization of $(1\rightarrow3)$-β-D-glucan in the tested home environments might not have reached the full potential quantified by the aerosolization-based microbial source assessment.

At the same time, the aerosolization ratio of $(1\rightarrow3)$-β-D-glucan calculated by relating the air to the dust concentration was higher in winter in most of the tested homes (71%), which can be explained by lower humidity in winter. In contrast to endotoxin, the $(1\rightarrow3)$-β-D-glucan levels in the floor dust and aerosolized fractions were mostly comparable, and 25% homes showed the aerosolizable level even higher than the dust-borne one. There were 9% of such homes in winter and 35% in summer. Similar to endotoxin, the lower humidity observed in winter has likely facilitated desiccation of $(1\rightarrow3)$-β-D-glucan-containing particles on surfaces, which enhanced aerosolization.

The geometric mean of $(1\rightarrow3)$-β-D-glucan concentration obtained from the dust samples in this study (winter and summer data combined) was 116 μg/m², which is more than 6-fold higher than the value obtained from the vacuum-collected floor dust samples in a 574-home study conducted in Cincinnati (geometric mean = 18 μg/m²; Iossifova et al., 2007). Thus, similar to endotoxin, dust-borne $(1\rightarrow3)$-β-D-glucan levels in flood-affected New Orleans homes remained considerably high, even though two years had passed since the flood.

The $(1\rightarrow3)$-β-D-glucan concentrations in air samples had a geometric mean of 2.71 ng/m³ for the entire data set (the summer and winter campaigns together). This level is lower than airborne concentrations of $(1\rightarrow3)$-β-D-glucan reported in previous studies conducted in flood-affected homes and close to the typical levels measured in non-flood-damaged homes. For example, Rao et al. (2007) found a geometric mean of the airborne $(1\rightarrow3)$-β-D-glucan and $(1\rightarrow6)$-β-D-glucan to be 1600 ng/m³ when sampling in flood-affected homes of New Orleans within three months after the floods; approximately one year after the floods, Reponen et al. (2007) found a geometric mean of $(1\rightarrow3)$-β-D-glucan to be much lower: 6.57 ng/m³. The geometric mean of airborne $(1\rightarrow3)$-β-D-glucan concentrations measured in non-moldy homes in Cincinnati were even lower: 0.92 ng/m³ (Lee et al., 2006), 1.0 ng/m³ (Crawford et al., 2009) and 2.0 ng/m³ (Reponen et al., 2009).

The levels of $(1\rightarrow3)$-β-D-glucan obtained in the summer and winter campaigns were also compared separately for homes treated with bleach and other chemicals versus non-treated homes using paired t-test. The strong statistically significant decrease from summer to winter was confirmed for each of the two sub-groups of homes based on both the dust ($n=8$) and FSSST ($n=9$) samples. Air samples ($n=7$), in contrast, showed no significant seasonal differences in $(1\rightarrow3)$-β-D-glucan levels in either sub-group.

3.3. Exposure matrices of fungi

Levels (mean ± SD) of total fungi in FSSST and air samples collected in summer were $(9.34\pm16.11)\times10^5$ spores/m² and $(3.03\pm2.55)\times10^3$ spores/m³, respectively. The corresponding values in winter were: $(4.38\pm8.86)\times10^5$ spores/m² and $(4.44\pm2.55)\times10^3$ spores/m³. The spore type specific data are presented in Table 2. No significant seasonal difference was determined for airborne total fungal spores similar to $(1\rightarrow3)$-β-D-glucan. This suggests that the fungal aerosolization in home environments did not reach the full potential and/or the aerosolized fungal spores (including agglomerates) were sufficiently large to be subjected to significant gravitational settling. However, most of the FSSST samples showed lower spore concentration during winter with the p-value obtained in paired t-test close to significance level ($p=0.063$). Mattress versus floor and chemically treated versus non-treated homes were not compared with respect to fungal spores because of the small sample size (only ~30% of samples were chosen for fungal spore analysis).

Researchers who measured airborne fungal spore levels within 2–6 months after the floods reported much higher concentration levels, ranging from 280×10^3 to 735×10^3 spores/m³ (Chew et al., 2006; Solomon et al., 2006; Rao et al., 2007; Schwab et al., 2007). Approximately one year after the floods, the indoor concentration of airborne fungi decreased, as evident from the study of Reponen et al. (2007) that reported a geometric mean of 12×10^3 spores/m³ (from three flood-affected homes). Previous studies in non-moldy homes in Cincinnati demonstrated the following geometric means of airborne spore concentrations: 573 spores/m³ (Lee et al., 2006), 214 spores/m³ (Crawford et al., 2009) and 120 spores/m³ (Reponen et al., 2009). Another study that included both non-moldy and moldy Cincinnati homes revealed a geometric mean of 145 spores/m³ (Osborne et al., 2006). We concluded that while the airborne spore concentrations in the flood-affected homes seem to have decreased after the initial water damage (the geometric mean of both the summer and winter data obtained in this study was 1.9×10^3 spores/m³), the levels were still slightly higher than those typically measured in homes not affected by major flooding.

The composition of predominant spore types had remained the same as observed by other investigators within 6 months of the floods. Similar to the observation of Solomon et al. (2006), we also found that the highest concentrations were of Aspergillus/Penicillium, Cladosporium, ascopsores among the fungal types identified in air samples. Chaetomium and Stachybotrys were identified in air samples occasionally in summer (see Table 2).

3.4. Exposure matrices of dust mite allergens

As stated in the Introduction section, we anticipated high levels of dust mite allergens in the flood-affected homes. However, detectable levels of at least one of the two allergens (Der p1 or Der f1) were found in only a few floor or mattress dust samples (from six homes sampled in summer and three homes sampled in winter). Overall, detectable levels of these allergens were found in dust samples collected in seven out of eight occupied homes and in two out of 27 unoccupied homes. All FSSST and air samples had the dust mite allergen values below the LLOD, even after concentrating the samples.

Table 2
Concentration of total fungal spores in FSSST and air samples during summer and winter.

	Summer				Winter			
	Fungal spores in FSSST samples (n=10)		Fungal spores in air samples (n=10)		Fungal spores in FSSST samples (n=8[a])		Fungal spores in air samples (n=10)	
	Range (spores/m²)	Percentage of occurrence (%)	Range (spores/m³)	Percentage of occurrence (%)	Range (spores/m²)	Percentage of occurrence (%)	Range (spores/m³)	Percentage of occurrence (%)
Spore types								
Alternaria	<LLOD–14,899	10	<LLOD–17	10	<LLOD–59,597	25		0
Aspergillus/Penicillium	39,110–1,013,144	100	100–3504	100	<LLOD–953,547	88	131–12,519	100
Arthrinium	<LLOD–104,294	10	<LLOD–130	20	<LLOD–119,193	13		0
Ascospores	11,174–208,589	100	<LLOD–1105	90	<LLOD–119,193	63	<LLOD–3130	80
Basidiospores	<LLOD–208,589	50	<LLOD–765	80		0	<LLOD–709	30
Bispora	<LLOD–5587	10	<LLOD–14	10		0		0
Botrytis	<LLOD–5587	10	<LLOD–8	10		0	<LLOD–14	10
Botryodiplodia		0	<LLOD–95	10		0		0
Cercospora		0	<LLOD–41	20		0	<LLOD–14	10
Coprinus		0	<LLOD–52	20		0		0
Chaetomium	<LLOD–2,771,247	50	<LLOD–945	70	<LLOD–595,967	25		0
Cladosporium	<LLOD–59,597	50	<LLOD–408	80	<LLOD–11,174	33	38–6203	60
Curvularia	<LLOD–23,349	20	<LLOD–8	10		0		0
Drechslera		0	<LLOD–95	30		0		0
Epicoccum	<LLOD–14,899	10	<LLOD–7	10		0		0
Ganoderma		0	<LLOD–389	80	<LLOD–59,597	13	<LLOD–39	10
Nigrospora	<LLOD–44,698	10		0		0	<LLOD–165	10
Periconia		0	<LLOD–378	10		0		0
Peronospora		0	<LLOD–95	20		0		0
Pithomyces	<LLOD–14,899	10		0		0		0
Polythrincium		0	<LLOD–8	10		0		0
Psathyrella		0	<LLOD–8	10		0		0
Rusts		0	<LLOD–25	20		0		0
Smuts/Myxomycetes		0	<LLOD–544	60	<LLOD–238,387	13		0
Sporidesmium		0	<LLOD–23	20		0	<LLOD–14	10
Stachybotrys		0	<LLOD–136	20	<LLOD–5587	13		0
Tetraploa		0	<LLOD–6	10		0		0
Unknown spores	5587–134,093	100	<LLOD–816	60	<LLOD–297,984	50	<LLOD–1521	40
Total spores	55,872–4,559,149		200–8128		27,936–2,443,465		232–15,649	

Abbreviation: LLOD: Lower limit of detection.
[a] n = 8 for winter FSSST samples because spores in two samples were not visible under the microscope due to excess of dust particles.

The concentrations of Der p1 and Der f1 in dust samples collected from floors and mattresses are presented in Table 3.

In general, it appeared that moisture damage did not promote dust mite population growth after the flood. In all three homes where dust mite allergen levels were measured both in the floor and mattress dusts, significantly higher levels of Der p1 and Der f 1 were found in mattresses (see Table 3), indicating that moisture-damaged mattresses could provide better niches for the dust mite populations. As shown in Table 3, these three homes were occupied and were not treated with bleach and other chemicals. Previously, we also found that the amounts of dust mite allergens aerosolized from different flood-affected materials collected in New Orleans homes were below the detection limit (Adhikari et al., 2009).

While relative humidity of 70–80% can promote the dust mite population growth, further increase in the humidity (above 85%) does not provide favorable growth conditions (Voorhorst et al., 1969; Spieksma, 1990). These "extreme" humidity conditions likely occurred immediately after the flood and probably diminished the existing dust mite populations. However, it is not clear whether this disappearance directly reflects the humidity effect or was caused by indirect ecological effects that enhanced growth of other microorganisms (e.g., fungi), which could have inhibited the growth of mite

Table 3
Dust mite allergen levels in dust samples collected during summer and winter. Only homes that had detectable levels of dust mite allergens are included.

Home ID	Floor material	Signs of water damage	Signs of visible mold	Occupation status	Bleach and other chemicals treatment status	Der p1 (µg/g) Floor	Mattress	Der f1 (µg/g) Floor	Mattress
Summer									
2234S	Carpet	Yes	Yes	Unoccupied	Treated	<LLOD	N/A	0.08	N/A
3605M	Hardwood	Yes	Yes	Occupied	Untreated	<LLOD	1.64	11.78	>ULOD
834J	Carpet	Yes	No	Occupied	Treated	1.78	1.65	13.51	>ULOD
1350SA	Linoleum	Yes	Yes	Occupied	Untreated	<LLOD	N/A	0.06	N/A
7141R	Hardwood	Yes	Yes	Unoccupied	Untreated	<LLOD	N/A	0.08	N/A
11011H	Hardwood	Yes	Yes	Occupied	Treated	<LLOD	N/A	0.07	N/A
Winter									
926M	Carpet	Yes	Yes	Occupied	Untreated	<LLOD	N/A	2.21	N/A
3605M	Hardwood	Yes	Yes	Occupied	Untreated	4.07	18.86	0.31	5.90
1350StA	Linoleum	Yes	Yes	Occupied	Untreated	<LLOD	N/A	0.145	N/A

Abbreviations: LLOD: Lower limit of detection; ULOD: Upper limit of detection.
Note: All other dust samples, FSSST samples, and air samples showed<LLOD levels for both Der p1 and Der f1.

population. We also anticipate that after the floods, mite re-infestation did not occur because the homes were unoccupied for a long time. Pike (1998) previously found that houses with a low number of occupants take a long time to become infested with dust mites and by inference, people must be occupying the house for infestation to occur, which was mostly lacking in the flood-affected homes. There is also the possibility that the allergens in the flood-affected materials were altered in some way that precluded measurement by ELISA. For example, dust mite allergens can be affected by tannic acid and other chemicals (Woodfolk et al., 1995; Chew et al., 1999).

3.5. Effects of temperature, relative humidity, and moisture levels on endotoxin and $(1 \rightarrow 3)$-β-D-glucan levels

The temperature levels in the investigated homes ranged from 26.40 to 42.75 °C (mean ± SD = 30.60 ± 3.03 °C) during summer and 13.8 to 34.7 °C (mean ± SD = 20.62 ± 4.82 °C) during winter. The relative humidity levels were 47.00 to 69.50% (mean ± SD = 61.97 ± 5.66%) during summer and 35.00 to 78.50% (mean ± SD = 49.98 ± 11.75%) during winter. Surface moisture was measured during sampling by a protimeter, and readings were qualitatively classified into 'dry', 'at risk', and 'wet' categories, corresponding to color changing LEDs in the protimeter showing green (dry), yellow (at risk) and red (wet) lights. According to the manufacturer, surface materials in the green zone are in safe air-dry condition; in the yellow zone, moisture levels are higher than normal, but not critical; further investigation is recommended; and the red zone represents excessive moisture levels. If sustained, red zone moisture levels can lead to decay in organic materials. A total of 11, 17, and 7 homes during summer, and 17, 11, and 1 home during winter were classified into 'dry', 'at risk', and 'wet' categories, respectively (no data were recorded in two homes due to an instrument problem). This suggests that moisture levels on the surfaces had decreased in the tested homes between summer and winter, but a considerable number of homes were still under 'at risk' category, even two years after the floods.

We calculated Pearson correlation coefficients between the log-transformed concentration levels of endotoxin, $(1 \rightarrow 3)$-β-D-glucan, and fungi, in one comparison, and the levels of temperature and relative humidity, as another comparison. The following statistically significant correlations were observed: (a) significant negative correlation between temperature and $(1 \rightarrow 3)$-β-D-glucan in dust ($r = -0.532$, $p < 0.05$) and FSSST ($r = -0.576$, $p < 0.05$) during summer only; (b) significant negative correlation between temperature and endotoxin in dust ($r = -0.644$, $p < 0.05$) during summer only; and (c) significant negative correlation between relative humidity and $(1 \rightarrow 3)$-β-D-glucan in FSSST only during winter ($r = -0.457$, $p < 0.05$). No significant associations were observed between the protimeter moisture level readings and the levels of endotoxin and $(1 \rightarrow 3)$-β-D-glucan. Negative correlations with temperature indicate that higher temperature did not facilitate the growth of specific fungal and bacterial species, which were prevalent in the flood-affected homes and contributed to a major portion of the endotoxin and $(1 \rightarrow 3)$-β-D-glucan levels.

3.6. Correlation between different exposure matrices

Because endotoxin and $(1 \rightarrow 3)$-β-D-glucan are potential immunomodulators for various atopic respiratory disorders, it is worthwhile to examine their correlation in indoor home environments. Their separate effects or combined synergistic effects can aggravate respiratory allergy and other pulmonary diseases in different ways, and little is known about this emerging research area. As the endotoxin level was mostly higher in winter months than in summer but $(1 \rightarrow 3)$-β-D-glucan demonstrated the opposite trend (higher in summer), we did not anticipate a correlation between the datasets combined for both seasonal campaigns. When seasonal data were

separately considered, we found a significant positive correlation between the endotoxin and $(1 \rightarrow 3)$-β-D-glucan in dust samples (Pearson correlation coefficient, $r = 0.535$; $p < 0.05$) and air samples ($r = 0.699$; $p < 0.05$) during summer, but not in winter. While assessing the aerosolization potential of sources through the FSSST sampling, we determined no correlation between endotoxin and $(1 \rightarrow 3)$-β-D-glucan. No significant positive correlations were observed between the levels of $(1 \rightarrow 3)$-β-D-glucan and fungal spores detected in FSSST and air samples. This lack of correlation can be attributed to various organic sources for $(1 \rightarrow 3)$-β-D-glucan in the sediments of flood water, other than fungi.

When the association between the levels of endotoxin and $(1 \rightarrow 3)$-β-D-glucan collected by three different methods were examined, we found a statistically significant positive correlation for $(1 \rightarrow 3)$-β-D-glucan between dust and FSSST samples only ($r = 0.28$; $p < 0.05$). This points to a similarity between $(1 \rightarrow 3)$-β-D-glucan on floor surfaces (available to become airborne by air movement) and inside floor materials (not readily aerosolizable). No similar correlation was observed for endotoxin. It is probably because endotoxin associated with bacterial growth remained primarily inside the floor material. The lack of correlation further underscores that using a single exposure assessment method may not be adequate for understanding the inhalation exposure risks associated with microbial contaminants.

3.7. Benefit of using different matrices of exposure: the present study versus other studies in flood-affected homes New Orleans

Unlike many similar investigations, our study design allowed combining long-term inhalable air sampling and conventional dust sampling with the assessment of potential aerosolization of biocontaminants from surfaces using a unique microbial source strength tester. Levels of airborne microorganisms determined by conventional air sampling generally serve as the direct and most reasonable way to assess the inhalation exposure. During specific time intervals, however, the air sampling data may not be a perfect representative of true exposures because of an inability to detect microbial colonization and successive microbial aerosolization from surfaces (Horner, 2003). The release of microbial contaminants from moisture-damaged surfaces may not occur during the collection of air samples. Furthermore, it may be sporadic, even with little or no disturbance of the surfaces. Previous researchers have used vacuumed floor dust as a surrogate for inhalation exposure because it is believed to be less influenced by short-term variability in indoor activities and ventilation and, therefore, more representative of long-term exposure than short-term air samples (Wickman et al., 1992). On the other hand, vacuuming can overestimate the inhalation exposure risks for the aerosolized microbial biocontaminants from flood-affected materials, as was concluded in this study as well as in our recently published study (Adhikari et al., 2009). To summarize, all currently available exposure assessment methods for airborne microorganisms have some affirmative features and some limitations. Although resource-wise it is not always feasible, assessing exposure matrices from different sampling methods (as conducted in this study) may provide an imperative benefit, particularly when environmental monitoring is performed in response to a major disaster with significant public health implications.

Previous bioaerosol exposure assessments in the flood-affected homes of New Orleans, conducted 2–6 months after Hurricanes Katrina and Rita, were primarily performed by one-time short-term air sampling and conventional dust sampling. Seasonal variation in concentrations of endotoxin and $(1 \rightarrow 3)$-β-D-glucan after the flood have never been investigated, although the data could be critical in determining the suitability of the affected homes for re-occupation. The present study utilized three different methods to quantify the microbial exposures through assessing the settled dust by vacuuming

(representing the reservoir for biocontaminants in homes) and by forceful aerosolization (representing maximum potential contamination of indoor air), as well as inhalable aerosol (representing inhalation exposure). As the field campaign included 35 homes during summer and 31 homes during winter, the sample size was significantly greater than in most of the previously published papers on microbial contaminants in homes that flooded during Hurricanes Katrina and Rita. The achieved results supplement the previous findings of Chew et al. (2006) and Rao et al. (2007), which used short-term sampling in investigating airborne concentration levels of endotoxin, glucans, and fungi in flood-affected homes of New Orleans. Long-term (24 h) and inhalable bioaerosol sampling generated in the present study using the Button Inhalable Sampler produced novel and significant information, given that inhalable aerosol sampling has not been conducted in other studies in flood-affected homes of New Orleans. Finally, we believe that, beyond providing information about biocontamination resulted from flooding events, the findings of this study will benefit relevant epidemiological investigations on biocontaminants and associated health effects.

3.8. Future potential research strategies based on our study findings

In this study, no conclusive relationships were found between the inhalable airborne levels of endotoxin, $(1 \rightarrow 3)$-β-D-glucan, and total fungal spores and their respective levels in dusts and FSSST samples. At the same time, biocontaminants that may be potentially aerosolized from floor and other surfaces can play an important role in influencing the inhalation exposure doses. Our findings suggest that a complex relationship exists between microbial contaminants, environmental factors and different activities. Further research on microbial ecology and diversity in moisture-damaged homes is necessary to understand this relationship, which is critical for the development of public health policies. Until this relationship is well characterized, investigating multiple exposure matrices has clear advantages over employing a single exposure assessment method. Additional scientific information can be gathered if microbial species in collected samples are identified in future prospective studies in flood-affected tropical homes and their ideal growth temperature conditions are determined. It appears that the effects of temperature, relative humidity, and moisture levels on endotoxin and $(1 \rightarrow 3)$-β-D-glucan are not straightforward in the complex niches of flood-affected homes. Follow-up studies on the ecological aspects of fungal and bacterial species in moisture-damaged homes will help interpret the associations between environmental factors and these biocontaminants. Further research is also needed to understand the effects of flood-water damage on mite population growth and allergen levels. Additional field monitoring can also allow evaluation of the time needed for the dust mite population to replenish to the pre-flood levels.

4. Conclusions

Elevated levels of endotoxin and $(1 \rightarrow 3)$-β-D-glucan were detected in the dust samples collected from the flood-affected homes in New Orleans about two years after the floods caused by Hurricanes Katrina and Rita. The concentrations of airborne fungal spores were also higher than in "normal" home environments, but exhibited considerable decreases during a two-year period after the initial water damage. Our findings indicate that collection of dust samples by vacuuming could considerably overestimate the inhalation exposure risks associated with endotoxin. While no significant difference was observed in the dust-borne endotoxin levels measured in summer and winter, the aerosolization of endotoxin was higher in winter than in summer in 71% homes, which was supported by the source aerosolization and air sampling data. The concentration of airborne endotoxin followed the trend determined for its aerosoliza-

tion potential. In contrast to endotoxin, the $(1 \rightarrow 3)$-β-D-glucan levels in the floor dust and aerosolized fractions were mostly comparable, and 25% of the homes showed the aerosolizable level even higher than the dust-borne one. The seasonal patterns for endotoxin in dust and the aerosolizable fraction were different from those found for $(1 \rightarrow 3)$-β-D-glucan. This reflects the difference in effects of the air temperature and humidity on bacterial and fungal contamination. While the concentration of airborne endotoxin followed the same seasonal trend as its aerosolization potential, no significant seasonal difference was identified for the concentrations of airborne $(1 \rightarrow 3)$-β-D-glucan, as well as for airborne fungal spores. Detectable dust mite allergens were only found in a few dust samples and not in FSSST and air samples, indicating negligible chance of exposures to Der p1 and Der f1 allergens in flood-affected homes two years after a major flood. No conclusive correlations were found between the three environmental monitoring methods. This disparity indicates that investigating multiple exposure matrices is more advantageous than employing a single exposure assessment method for microbial contaminants in moisture-damaged homes.

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Molds and mycotoxins in dust from water-damaged homes in New Orleans after hurricane Katrina

Abstract Dust collected in New Orleans homes mold-contaminated because of the flooding after hurricane Katrina was analyzed for molds and mycotoxins. The mycoflora was studied by cultivation and quantitative PCR for selected molds. The most commonly found mold taxa were *Aspergillus, Cladosporium,* and *Penicillium*. Verrucarol, a hydrolysis product of macrocyclic trichothecenes predominately produced by *Stachybotrys* spp. was identified in three dust samples by gas chromatography-tandem mass spectrometry, and sterigmatocystin (produced by various *Aspergillus* spp.) was found in two samples by high pressure liquid chromatography-tandem mass spectrometry. This is the first demonstration of mycotoxins in Katrina-associated dust samples. The analytical methods used represent valuable tools in further studies on bioaerosol exposure and health risks.

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Practical Implications
In the aftermath of natural disasters like hurricane Katrina water-damages on infrastructure and public and private property are often associated with health risks for remediation workers and returning residents. In the case of New Orleans evaluations of health hazards, health studies, and assessments of bioaerosol have been conducted previously. However, until now mycotoxins have not been addressed. Our study shows, for the first time, the presence of mycotoxins in dust collected in houses in New Orleans mold-contaminated because of the hurricane Katrina. The results may highlight the potential health threats posed by mold aerosols in post-disaster inhabited areas.

Introduction

In August 2005, hurricane Katrina, one of the costliest and deadliest natural disasters in the history of United States, struck Louisiana, Mississippi, and parts of Florida, Georgia, and Alabama. In New Orleans, the city levee system catastrophically failed; about 80% of the city flooded to varying depths of up to about 6 m, causing a large loss of lives and property damage (Knabb et al., 2005). Because of the floods, approximately 80% of all houses and their contents were heavily contaminated by mold growth. In this study the objective was to analyze house dust samples collected in water-damaged buildings in New Orleans for the presence of molds and some selected

mycotoxins (toxic secondary metabolites of molds) potentially produced in damp indoor environments (Bloom et al., 2007b). Verrucarol (VER) and trichodermol (TRID), hydrolysis products of macrocyclic trichothecenes (including satratoxins) and trichodermin respectively (all predominately produced by *Stachybotrys* spp.), were analyzed by gas chromatography-tandem mass spectrometry (GC-MSMS). Sterigmatocystin (STRG, mainly produced by *Aspergillus* spp.), satratoxin G (SATG), and satratoxin H (SATH) were analyzed by high pressure liquid chromatography-tandem mass spectrometry (HPLC-MSMS). The dust mycoflora was studied by cultivation and by a mold-specific quantitative PCR assay (MSQPCR) (Haugland et al., 2002).

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Materials and methods

Chemicals and standards

Solvents and reagents were of analytical or HPLC grade and used without any further purification. Buffers were degassed and filtered through 0.45 μm filters (Millipore, Bedford, MA, USA) before use, and water was distilled and deionized. Cultivation media were prepared at the Department of Laboratory Medicine in Lund according to protocols provided by the Centraalbureau voor Schimmelcultures, the Netherlands (http://www.cbs.knaw.nl/). All cultivation media were supplemented with 100 ppm chloramphenicol (Sigma, St Louis, MO, USA). Methanol, dichloromethane, sodium chloride and sodium hydroxide were purchased from Fischer Chemicals (Leicester, UK) and acetonitrile, toluene, and acetone from Lab Scan (Dublin, Ireland). N-heptafluorobutyrylimidazole (HFBI), STRG, and VER were purchased from Sigma (Schnelldorf, Germany). 1,12-Dodecanediol, ammonium acetate, and sodium acetate were purchased from Fluka (Schnelldorf, Germany and Bachu, France). Reserpine (5 ng/μl) was purchased from Varian Inc. (Walnut Creek, CA, USA). Trichodermin was a kind gift from Poul Rasmussen (Leo-pharma, Ballerup, Denmark), and TRID was derived from trichodermin by hydrolysis. Crude SATG and SATH mycotoxin standards were kindly provided by Professor Bruce B. Jarvis (Department of Chemistry & Biotechnology, University of Maryland, College Park, MD, USA).

Sampling

The field sampling was conducted during January 2007 in five severely mold-contaminated single-family houses (Table 1) in New Orleans, LA, USA, which had been flooded during hurricane Katrina. Sampling was made approximately 5 months after flood water receded; at that time the houses had not been remediated or re-inhabited. Bulk composite samples were collected in small plastic bags from the middle of the floor surface area of selected rooms using a small plastic brush and dust pan (washed with soap and water, sprayed with 70% ethanol, and dried before using in each home), and kept at −20°C before analysis.

Cultivation and identification

Approximately 100 mg of each dust sample was suspended and serially diluted (10^1, 10^2, 10^3, 10^4, 10^5 times) in 0.85% NaCl solution. An aliquot from each dilution was plated onto malt extract agar, czapek yeast extract agar, and dichloran 18% glycerol agar, and incubated at 22°C for 7 days. Colony forming units were counted and fungal taxa identified using phase-contrast microscopy according to Samson et al. (1995).

Quantitative PCR

Parts of the dust samples (10 mg) were sent to Anozona (Uppsala, Sweden), contract partner of Fugenex (Yorkshire, UK), who are licensed to use DNA extraction and MSQPCR analysis according to Haugland and Vesper (2002) (US patent 6,387,652) as described elsewhere (Haugland et al., 2002; Vesper et al., 2004). Information on the primer and probe sequences is provided at http://www.epa.gov/microbes/moldtech.htm.

Sample preparation, extraction and purification

Dust (~1 g) was prepared for chemical analysis (Bloom et al., 2007a,b). In brief, samples were extracted with methanol (2–3 ml) in 10-ml glass test tubes with Teflon-lined screw caps, stored in the dark for 48 h at room temperature, and centrifuged. The supernatants were decanted into new tubes. Extraction was repeated with dichloromethane after which the methanolic and dichloromethane phases were pooled, evaporated under a gentle stream of nitrogen, dissolved in dichloromethane, and applied onto polyethyleneimine

Table 1 Description of five flooded homes in New Orleans from which bulk dust samples were collected

	House 1	House 2	House 3	House 4	House 5
Sample Date	1/8/2007	1/8/2007	1/8/2007	1/29/07	1/25/2007
Amount of flood water	~3 m	~3 m	~3 m	~3 m	~1.5 m
Type of building	Single	Single	Single	Single	(1/2) Attached Double
	Bricked, ranch style	Bricked, ranch style	Bricked, ranch style	Bricked, ranch style	Raised wood frame, shotgun style
	Concrete floor	hardwood, tile, terrazzo, and area rug flooring	Tile, concrete floors	Carpet and concrete flooring	Hardwood, linoleum, and carpet flooring
	Living, kitchen, bath, (3) bedrooms	Living, kitchen, bath, (3) bedrooms	Living, kitchen, bath, (3) bedrooms	Living, kitchen, bath, (3) bedrooms	Living, kitchen, bath, (2) bedrooms
Samples collected	Combined bulk sample	Combined bulk from hardwood, tile and walls; bulk from tile and hardwood	Combined bulk from tile and concrete	Bulk from concrete	Bulk from linoleum and hardwood

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bonded silica gel columns (PEI, 1 ml, JT Baker, Phillipsburg, NJ, USA). Samples were eluted with 5 ml dichloromethane, evaporated, re-dissolved in 0.5 ml methanol, and filtered through 0.45 μm Millex syringe filters (PTFE, Millipore, Bedford, MA, USA) into new Teflon-capped analysis vials. After adding 1 ng of reserpine as an internal standard, 50 μl was taken for direct HPLC-MSMS analysis. The remaining 450 μl were mixed with 500 pg 1,12-dodecanediol (internal standard in GC-MSMS), evaporated, hydrolyzed in 0.2 M methanolic NaOH, and extracted with water and dichloromethane. The organic phase extracts were taken to new tubes, evaporated to dryness and placed in a desiccator overnight. The dried extracts were then subjected to derivatization by adding 80 μl of acetonitrile-toluene (1:6, v:v) and 20 μl of HFBI, followed by heating at 70°C for 60 min. Samples were then left standing in excess of derivatizing agent at room temperature at minimum of 4 h before analysis by GC-MSMS.

HPLC-MSMS and GC-MSMS

A ProStar HPLC/1200L triple quadrupole MSMS system (Varian Inc., Walnut Creek, CA, USA) was used. 20 μl of sample was injected (in duplicate) using an autosampler (Varian, model 410) into a Polaris 5 μM C18-A 150 × 2.0 mm RP-18 column. An initial methanol concentration of 20% was held for 1 min, after which it was raised linearly (9 min) to 70% before it was again raised linearly (10 min) to 100% and held for 9 min. At the end of the run, the concentration of methanol was linearly lowered again (1 min) to 20% and kept 8 min for stabilization. See (Bloom et al., 2007b) for further details.

Derivatives were analyzed by GC-MSMS using a CP-3800 GC triple quadrupole MSMS system (Varian Inc.). Analyses were made in negative chemical ionization mode, at 70 eV, an ion source temperature of 150°C, and with ammonia as ionization gas (0.4 kPa). Duplicate sample volumes of 1 μl were injected onto a

FactorFOUR™ fused-silica capillary column (VF-5 ms, 30 m × 0.25 mm i.d) (Varian Inc.) in the splitless mode. A mix of HFBI and acetone (1:3, v:v) was injected in between samples to eliminate any trace of un- or semi-derivatized VER/TRID. Details on MSMS conditions are provided elsewhere (Bloom et al., 2007a,b).

Results

Cultivation

The mycoflora of the cultivated dust samples is described in Table 2. House 1 and House 3 contained the highest number of fungal propagules and House 4 the lowest. Indicator organisms of water damage, e.g. *Chaetomium* sp., *Trichoderma* sp., and yeasts, were identified in approximately half of the samples. Only one sample (House 4) did not show any growth of *Aspergillus* spp., and *Stachybotrys* spp. were not found in any sample.

PCR

The results are summarized in Table 3. As in the cultivation studies House 1 and House 3 contained the largest amounts of fungi and House 4 the least. *Stachybotrys chartarum* was identified in all samples

Table 3 Number of mold and bacterial DNA-sequences per mg dust

Sample	Total mycoflora	A. versicolor	S. chartarum	Aspergillus spp. Penicillium spp.	Streptomyces spp.
House 1	716,000	n.d.	7972	7160	448
House 2a	10,040	n.d.	14	237	n.d.
House 2b	67,760	n.d.	13	2087	n.d.
House 2c	21,168	n.d.	66	117	n.d.
House 3	1,353,200	n.d.	572	11,824	n.d.
House 4	385	n.d.	n.d.	n.d.	n.d.
House 5	159,120	27	36	253	n.d.

n.d., not detected.

Table 2 Molds cultured from the dust samples using three different agar media

Sample	CFU/mg dust (Fungal taxa)		
	MEA	CYA	DG18
House 1	1280 (Penicillium, Aspergillus, Trichoderma, yeast, Cladosporium, Mycelia sterila)	9700 (Aspergillus, Trichoderma, Cladosporium)	922 (Penicillium, Aspergillus, yeast, Mycelia sterila)
House 2a	0	53 (Cladosporium, Aspergillus, Mycelia sterila)	490 (Penicillium, yeast, Mycelia sterila)
House 2b	55 (Cladosporium, Aspergillus, Chaetomium)	100 (Cladosporium, Aspergillus, Mycelia sterila)	18 (Aspergillus, Paecilomyces)
House 2c	85 (Aspergillus, Cladosporium, Mycelia sterila)	60 (Cladosporium, Aspergillus, bacteria)	10 (Mycelia sterila)
House 3	20580 (Aspergillus, Trichoderma, yeast, Cladosporium, Chaetomium, Alternaria, Fusarium, Mycelia sterila)	13880 (Aspergillus, yeast, Trichoderma, Cladosporium, Chaetomium, Mycelia sterila)	20870 (Penicillium, Trichoderma)
House 4	0	0	100 (Yeast, Mycelia sterila)
House 5	9 (Aspergillus)	61 (Penicillium, Alternaria, Mycelia sterila)	9 (Aspergillus)

CFU, colony forming units; Mycelia sterila, non-sporulating mycelium; MEA, malt extract agar; CYA, czapek yeast extract agar; DG18, dichloran 18% glycerol agar.

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Table 4 Mycotoxins in the studied dust samples

Sample (~1 g dust)	Pg mycotoxin/mg dust				
	TRID	VER	STRG	SATG	SATH
House 1	n.d.	0.6	16	n.d.	n.d.
House 2a	n.d.	n.d.	n.d.	n.d.	n.d.
House 2b	n.d.	n.d.	n.d.	n.d.	n.d.
House 2c	n.d.	n.d.	28	n.d.	n.d.
House 3	n.d.	n.d.	n.d.	n.d.	n.d.
House 4	n.d.	0.6	n.d.	n.d.	n.d.
House 5	n.d.	18	n.d.	n.d.	n.d.

n.d., not detected.
TRID, trichodermol; VER, verrucarol; STRG, sterigmatocystin; SATG, satratoxin G; SATH, satratoxin H.

except House 4 where also the other specified fungal species/taxa and *Streptomyces* bacteria were absent. *Aspergillus versicolor* was only detected in House 5 and *Streptomyces* only in House 1.

Mycotoxin analysis

The mycotoxin findings are summarized in Table 4. VER was found in House 5 (18 pg/mg dust) and in trace amounts in House 1 and House 4 (0.6 pg/mg dust). In these analysis ions of m/z 638 (M − HF) were fragmented and quantification was performed based on product ions of m/z 213 as described earlier (Bloom et al., 2007a). STRG was found in House 1 (16 pg/mg dust) and in House 2c (28 pg/mg dust). Here, ions of m/z 325 were fragmented and product ions of m/z 310 were used for quantification (Bloom et al., 2007b). TRID, SATG, and SATH were not found in any of the dust samples. The limits of detection for VER, TRID, and STRG standards are 72, 37, and 19 pg added to 1 mg of dust respectively (Bloom et al., 2007a,b).

Discussion

There have been several reports concerning public health-related issues in post hurricane New Orleans including initial microbiological and chemical contaminant assessments, e.g. of semi-volatile compounds, arsenic, lead, and fecal indicator microorganisms (Pardue et al., 2005; Presley et al., 2006; Schwab et al., 2007; Sinigalliano et al., 2007). In microbial assessment studies in different locations high mean indoor/outdoor mold spore ratios were found; 4.11 (Schwab et al., 2007), 4.8 (Solomon et al., 2006), and 8.3 (Rao et al., 2007). The mold genera most commonly found indoors were, respectively, *Aspergillus* and *Penicillium*, *Cladosporium*, and *Curvaria* (Schwab et al., 2007), *Cladosporium*, *Aspergillus/Penicillium*, and *S. chartarum* (Solomon et al., 2006), *Aspergillus niger*, *Penicillium* spp., *Trichoderma*, and *Paecilomyces* (Rao et al., 2007), *Penicillium*, *Aspergillus* and *Paecilomyces* (Chew et al., 2006), and *Penicillium* and *Aspergillus* [Center for

Disease Control (CDC)., 2006]. Molds and fungal glucans were detected at highly-elevated concentrations (Rao et al., 2007) and the mycoflora was not typical for non-water damaged buildings (Rao et al., 2007; Solomon et al., 2006). Interestingly, the area concentrations of molds on indoor surfaces were consistent with measured airborne mold levels (Rao et al., 2007; Schwab et al., 2007), and after remediation intervention mold and endotoxin levels in the indoor air tended to be lower (Chew et al., 2006). In a CDC report from October 2005, glucan and endotoxin levels were significantly correlated and highest indoors (CDC, 2006). Highly-elevated concentrations of endotoxin were also found by Rao et al. (2007). However, Solomon et al. (2006) found no association between flooding and endotoxin concentrations or between endotoxin concentrations in flooded and non-flooded areas in New Orleans.

The fungal taxa found in the present work (except for yeasts, and in one case, *Fusarium*) have been identified also in other studies on molds associated with hurricane Katrina (as mentioned before) (Chew et al., 2006; Rao et al., 2007; Schwab et al., 2007; Solomon et al., 2006). Chew et al. (2006) used PCR for detecting 23 mold species in air samples before, during, and after renovation of three New Orleans homes; *Aspergillus*, *Penicillium*, and *Cladosporium* were the most common molds, and the prelevance of *Stachybotrys* was 40%. In our study, *Stachybotrys* was identified in all samples but one when using PCR, but not in a single sample when using culture. However, spores of *Stachybotrys* spp. in indoor environmental samples are often non-viable (Miller et al., 2000). *Aspergillus/Penicillium* species were found in all studied samples, with both PCR and culture, except in one. This sample (House 4) was PCR-negative for the studied microflora, and culture revealed only little growth of yeast and non-sporulating mycelium, even so traces of VER were detected (Table 4). These seemingly conflicting results may be explained by (i) the sample had not been thoroughly homogenized (*viz*, sub-samples used for toxin analysis and PCR may not be representative for the sample as a whole), (ii) a producer of VER or macrocyclic trichothecenes other than *Stachybotrys* was present (for example *Myrothecium* sp.), (iii) the sample was too diluted to contain enough DNA for PCR or, (iv) PCR inhibitors were present. In addition, the homeowner of house 4 may have applied antimicrobial agents, a common practice in post-Katrina New Orleans. Several such agents contain oxidizing substances e.g. chlorine and ozone known to degrade DNA.

Neither SATG nor SATH were found, not even in the VER-positive samples, which is likely to be due to the limited analytical sensitivity for these compounds in HPLC-MSMS in comparison with VER detection using GC-MSMS (Bloom et al., 2007b). The trichodermin-negative results may indicate that the

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