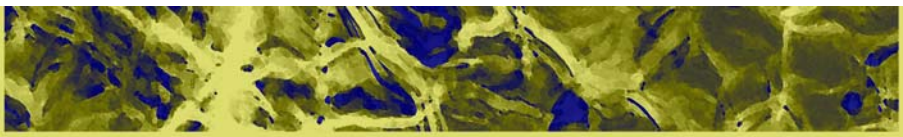

Policyholders of America

Research Committee Report on Diagnosis and Treatment of Chronic Inflammatory Response Syndrome Caused by Exposure to the Interior Environment of Water-Damaged Buildings

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Overview:

The Mold Research Committee presents our position statement on the current state of the science regarding human health effects acquired following exposure to the multiple microbes and microbial contaminants and their metabolites found in the interior environment of water-damaged buildings (WDB). These contaminants include but are not limited to fungi, bacteria, actinomycetes, and mycobacteria and their toxins; as well as inflammagens from fragments of fungal structures; and beta glucans, mannans, hemolysins, proteinases, spirocyclic drimanes and microbial volatile organic compounds (VOCs).

Exposure to these WDB environments can cause a readily identifiable illness syndrome characterized by specific metabolic disturbances stemming from lack of neuropeptide control of host inflammatory responses, genetic susceptibility and abnormal downstream inflammatory parameters that not only define the illness but also provide the academic basis for sequential therapeutic interventions.

Our findings are focused on abnormal physiologic changes in health, documented by health care professionals who are actively involved with case management of those made ill from exposure in WDB. The current body of literature describing the multiple adverse health effects acquired following exposure to the interior environments of WDB is extensive. The physiology unveiled by such literature mirrors the physiologic findings in chronic inflammatory response syndromes. The literature we cite throughout this document supports the concept that exposure to the interior environment of WDB creates abnormal inflammatory responses that are both biomarkers for the presence of the illness and guides for therapy. We note that the readily recognized abnormalities in host immune responses are initiated both by (i) the adverse effects of toxins such as mycotoxins, endotoxins and toxins made by actinomycetes; and (ii) effects of inflammagens. As the many possible sources of compounds that are found in WDB can each lead to the diversity of host responses seen, it is impossible to distinguish a unique source of such abnormal immune responses in patients with illness acquired following exposure to the interior environment of WDB.

Several consensus statements have been composed in the past decade. Yet none have included (a) assessments made by physicians involved with diagnosis and treatment of these adverse health effects; (b) academic papers written by physicians reporting both baseline and treatment data on the human illness; (c) reporting of results from published studies using treatment protocols or studies on prospective human or animal experimentation; (d) reporting based on objective parameters found in affected patients; and (e) clinical status after interventions. Despite these substantial shortcomings of pertinent information, these prior consensus statements are being used (i) in legal matters to report the state of human health effects from exposure in WDB and (ii) to serve as the basis for public health policy.

A more current reporting of accepted science that is based on literature review and includes, but is not limited to, experience of actual treating physicians is the foundation for our consensus opinion.

As identified by the US Government Accountability Office (GAO, 2008) report and the World Health Organization report (WHO, 2009), there are many compounds, both toxigens and inflammagens, present in the indoor air of a WDB that have been identified within the complex mixture found in the air and in the dust of the interior environments of WDB. Further, there is clear data showing that each of these compounds can initiate an inflammatory host response such that no single compound can be identified as the sole cause of the inflammatory responses seen in affected patients. Since many sources of inflammatory stimulus exist, some of which are synergistic, and no single causative agent within the WDB can be deemed to be solely responsible for the symptoms exhibited, the sole causative agent becomes the interior environment of the WDB itself. It is our consensus opinion that this syndrome acquired after exposure to water damaged buildings with evidence of amplification of microbial growth shall be referred to as, "Chronic Inflammatory Response Syndrome acquired following exposure to the interior environment of Water-Damaged Buildings (CIRS-WDB)."

We note in recent years a dramatic increase in published studies from the private sector, US governmental agencies and international health agencies with a focus on various and diverse human health effects acquired following exposure to the interior environment of WDB. These substantive papers support the understanding that:

(1) CIRS-WDB is a multisystem, multisymptom illness acquired following exposure to the interior environment of WDB and it exists as a recognizable syndrome. When defined by i) exposure; ii) symptom evaluation; and iii) epidemiologic similarities between studies of similar hosts and similar exposures, CIRS-WDB is both identifiable and treatable. A proven and consistent pattern of symptoms is demonstrated among published research findings involving both animal and human studies.

(2) CIRS-WDB is identified as immunologic in origin, with differential inflammatory responses seen according to (i) genetic susceptibility and (ii) unique aspects of host innate immune responses. Direct effects of microbial toxins, particularly mycotoxins, in pathogenesis are recognized to act synergistically with those toxins made by actinomycetes, gram negative bacteria, and possibly mycobacteria causing the effects shown in CIRS-WDB. Cellular immunity affecting T-cells and Th-17 plays a role in CIRS-WDB, as do immunologic changes activated by both toxins and inflammagens that are found in the interior environment of WDB. It is scientifically demonstrated that innate immune host responses are similar in their appearance following human exposure to many of the toxins and inflammagens that are simultaneously found to be present inside WDB. Documenting those immune abnormalities will not (and cannot) implicate any one, isolated, specific source. Given the current scientific information and readily available physiologic abnormalities that patients with CIRS-WDB experience, we must expand our assessment beyond the known effects of simple, individual toxins when establishing public health policy and private sector physician treatment protocols.

(3) CIRS-WDB consistently involves (but is not limited to) abnormalities in levels of regulatory neuropeptides MSH and VIP; pro-inflammatory cytokines IL-1B, IL-6, 8, 12, 13 and others; split products of complement activation, especially C4a; responses of hypoxia inducible factor, including but not limited to vascular endothelial growth factor (VEGF), erythropoietin and transforming growth factor beta-1 (TGF B-1); and cellular immunity. This type of cellular immunity includes effects on T-regulatory cells; Th-17 immunity impacting IL-17 and IL-23 functions; and auto-immunity, primarily anti-gliadin and anti-cardiolipin antibodies. Additional problems commonly seen are hormonal dysregulation involving corticosteroids (identified when ACTH and cortisol measured simultaneously), regulation of body osmolality (identified when ADH and osmolality measured simultaneously) and androgens; and coagulation factors, especially those represented by von Willebrand's profile. These laboratory findings are typical of those seen in other forms of CIRS.

(4) Treatment of human illness that is acquired following exposure to the interior environment of WDB is necessarily sequential. No single intervention is likely to correct all the underlying abnormalities in the inflammatory responses. Many approaches to treatment of CIRS-WDB are in current use. To date there has been a paucity of academic papers published on the entire selection of therapies used with success by individual practitioners.

(5) Taken as a whole, CIRS-WDB is a chronic inflammatory response syndrome, resulting from exposure to WDB and is readily identified by current methods of clinical diagnoses, with thorough differential diagnosis the key to linking the abnormal physiology seen to the cause of the illness. This process of diagnosis is supported by (i) identification of unique subsets ("clusters") of symptoms found in epidemiologic cohorts of affected patients; (ii) identification of unique groupings of biomarkers, such as genetic markers, neuropeptides, inflammatory markers, and autoimmune findings. The required tools of differential diagnosis include showing what patients *don't have* as well. Many tests commonly used in day-to-day medical practice, i.e., sedimentation rate, c-reactive protein, lipid profiles, thyroid studies, immunoglobulin studies (including IgE), metabolic profiles and complete blood counts are nearly always normal in CIRS-WDB. They are therefore of little to no value other than a pertinent negative finding in diagnosis of CIRS-WDB serving to rule out other potential causes of chronic symptoms. Regained health in response to sequential comprehensive therapy, published previously, that is founded on the understanding that there is a multiplicity of causative elements is an additional element that affirms the importance of differential diagnosis on an ongoing basis.

(6) We recognize that chronic and recurring systemic inflammation underlies the illness parameters of symptoms, laboratory findings, and neurotoxicological studies seen in the patients of clinical studies. These inflammatory processes mirror the experimental findings in animal models. We note that patients with CIRS-WDB are often given incorrect diagnoses such as depression, stress, allergy, fibromyalgia, Post Traumatic Stress Disorder, and somatization. Those conditions when actually present *will not improve* with therapies employed in CIRS-WDB. The regained health and improvement of objective laboratory parameters following appropriate treatments aimed at CIRS-WDB

demonstrate an additional clinical basis to distinguish CIRS-WDB from other diagnoses. This response to targeted therapy adds weight to the body of evidence that there is a multiplicity of toxigens and inflammagens that are causing a treatable chronic, recurring illness.

(7) CIRS-WDB is acquired primarily from inhalation of microbial products that are contaminants found in the complex mixture of the interior environment of WDB. We are aware that serious health problems, including fatalities, arising from ingesting kilograms of microbial-contaminated foods in developing countries are documented. Dermal contact and ingestion of settled WDB contaminants might theoretically contribute to the exposure burden of CIRS-WDB, but in reality, the quantities necessary to be harmful are unlikely to apply to daily life of patients in an indoor environment.

(8) Re-exposure of previously affected patients will bring about immunological host responses that are enhanced in their rapidity of onset and magnitude, such that these patients are "sicker, quicker." Without protective immunity being demonstrated either by laboratory methods or clinically, and even though they may have been treated adequately, those with prior CIRS-WDB must avoid contact with the interior environment of any WDB until such time as their abnormal reactivity to the multiplicity of causative agents found in the WDB can be either reduced or restored to pre-illness states. For some patients, such complete restoration is unattainable. As the state of current science dictates, those with especially severe reactivity to the interior of WDB must avoid re-exposures for the remainder of their lives to sustain their individual level of optimum health.

The study on acquisition of human illness following exposure to the interior environment of WDB includes infectious disease. The incidence of filamentous fungal infection is rising rapidly in both immuno-compromised and immuno-competent patients. There is a robust literature on fungal sinusitis leading to chronic rhino-sinusitis based on inflammatory changes induced by organisms such as *Alternaria, Aspergillus, Cladosporium* and *Penicillium*. Further, the role of fungal organisms in acquired immune illnesses, such as allergy, asthma, and hypersensitivity pneumonitis is also well described. This position statement accepts that these facts have been confirmed by a robust literature and no additional comment on these illnesses is indicated herein.

The weight of evidence is also overwhelming that respiratory infections, dermal infections, sinus-based inflammation, hypersensitivity pneumonitis and allergy are well understood aspects of illness that are each being adequately addressed in public policy and rapidly advancing physician treatment protocols.

This report will focus on the role of systemic inflammation and neurotoxicity as cardinal elements of CIRS-WDB.

The diverse groups of microbes found in WDB, including fungi, bacteria, actinomycetes and mycobacteria individually and collectively manufacture toxins

(including mycotoxins) and inflammagens on an ongoing basis. These compounds can individually and collectively activate innate immune inflammatory responses enhanced by their synergistic interaction within WDB. Current accepted science and diverse areas of research, individually and collectively, demonstrate the vast significance of the multiple microbial contributors found within WDB to human chronic and systemic inflammation resulting from the compounding synergy of the exposure. Abundant literature demonstrates (i) direct adverse effects of the toxins and inflammagens on animals and humans; and (ii) direct inflammatory effects of the antigens on animals and humans. Response to encompassing treatment for the complex mixture of exposure in the form of regained health validates the growing body of literature that the *mixture* itself is the underlying causative agent.

Current public health research needs include (i) creation of an HIPAA compliant, de-identified, national data base of patients identified by practitioners that can be accessed by collaborating researchers in the private sector and government agencies; (ii) development of a standard protocol for therapy based on the results of collaboration of actual practicing physicians; (iii) search for newer therapies based on genomics testing. Ongoing illness acquisition creates an opportunity for scientific advancements in diagnosis, therapy and prevention. Assessments of differential gene activation (genomics), with a link to assessment of compounds found in blood (proteomics), coupled with positive results of therapies based on these assessments show great promise in this field. Effective treatments in and of themselves serve as evidence of the cause of illness.

A method of disseminating current, ongoing and accurate information to medical teaching facilities regarding these illnesses and appropriate treatments must be established as a matter of public health policy.

This document describes the current state of the science behind CIRS-WDB provided from the perspective of the treating physician. It is our position that the extensive collaboration among individuals and organizations with direct experience with CIRS-WDB has provided a scientific basis to bring about a change in approach to evaluation and treatment of patients with exposure to the interior environment of WDB. We are grateful for the opportunity to have reviewed the literature and received feedback from academic researchers, practicing clinicians and especially patients commenting on WDB over the years. All written comments in this statement, however, come from the authors.

Background:

Health problems have been associated with exposure to WDB since Biblical times (Leviticus 14:34-47: The Bible, King James version, Oxford 1888). Nonetheless there was little peer reviewed literature that implicated inhalation of bioaerosols as causative of human illness before the 1970's in the United States. Several theories have emerged to explain the "new phenomenon" of buildings as a source of illness. Use of paper-faced gypsum board as wall coverings instead of plaster and lath saved time and reduced costs, but provided a wonderful source of food for indoor-dwelling microbes. Increasing use of flat roof construction, insulation and synthetic stucco surfaces each added to the potential for water intrusion into and through the building envelope of new construction. A change in recommendations for air turnover may have played a role as well in reduction of exchange of indoor air for outdoor air. Demand for new housing and increasing availability of heat pumps made use of crawlspaces as sites of ductwork for forced air delivery into vents on the first floor. Environmental conditions in crawlspaces, with a constant supply of moisture either from evaporation from the soils or condensation of warmer outside air against cool, earth temperature surfaces on interior surfaces in crawlspaces creates an ideal ecological niche for microbial growth. If the connections between segments of vent material, usually galvanized metal, fiberboard or flexible circular duct material, are not secured from air infiltration, over time the potential for distribution of microbes and their metabolites throughout the home is assured. Changes in use of HVAC equipment, with return air ducts taking air from crawl/ceiling spaces and basements directly into heating and cooling systems may also have contributed to the circulation of organisms and fragments of organisms from these spaces to the rest of the interior of residences, schools and commercial structures. Indirect evidence suggests that genetic changes in indoor filamentous fungi may also have occurred with use of fungicides in paint beginning in the 1970's.

Additional changes in construction methods took place as attempts to conserve energy and to promote energy efficiency. Efforts to make buildings more airtight in the 1970's led to increased use of re-circulated air in new construction. This resulted in reduced intrusion of exterior air through windows and doors. As costs of construction materials from natural sources increased, synthetic products such as oriented strand board (OSB) were cheaper to use than plywood. Unfortunately, there was often greater moisture retention if the sheets of OSB were stacked outside a job site and allowed to become wet before installation. Moreover, even if dry when installed, these man-made materials easily wick moisture if present from leaks and floods. High rise construction techniques also contributed to the increasing incidence of WDB. For example, concrete takes months to fully cure. However, as soon as the concrete floors and columns are firm enough to support addition of the next structure, the concrete is enclosed. This process unfortunately provides a reservoir of moisture to interior structures for months that would otherwise have remained dry. Added to this potential for interior water intrusion is the absence of moisture barriers between soil and concrete/masonry used in slab, foundations and basement walls.

Review of Current Agency Opinions (IOM, GAO, WHO and NIOSH):

Two recent publications have added great weight to the previously expressed opinions of practicing physicians who treat the human illness acquired following exposure to the interior environment of WDB. First is the U.S. GAO report published in September 2008, "Indoor mold: Better coordination of research on health effects and more consistent guidance would improve federal efforts" and second is the 2009 World Health Organization (WHO) report. Each of these publications notes the diversity of sources of pathophysiological inflammatory responses in CIRS-WDB and emphasizes the role of inflammatory immunologic responses in the illness.

While physicians knew from clinical observation that genetic associations and inflammatory abnormalities dominated clinical case presentation, government agencies have been slow to recognize these important features. The following discussion will highlight guidelines as put forth by the four major government agencies tasked with developing legal definitions and standards regarding indoor air quality (IAQ) as it impacts the health of its citizens. We will also address the urgent need for frequent and comprehensive revisions to accurately reflect the rapidly evolving research and clinical data as it becomes available.

Recognition of Health Effects by the Institute of Medicine (IOM):

A prime example of the importance of incorporating insights from new data into position statements leading to policy development can be seen by reviewing the 2004 publication from the Institute of Medicine (IOM). This document, commissioned by the Centers for Disease Control and Prevention (CDC) to reflect data from papers published before 2004, mentioned inflammation (cytokines) only thirteen times and genetics only eleven times. Of greater concern, the IOM did not acknowledge the key involvement of cytokines in the development of multiple symptoms in multisystem illnesses, simply noting their role in respiratory conditions as if systemic cytokine responses would affect only one organ system. More current and well-established research in animals and humans documents the involvement of cytokines as one element of the host inflammatory response following antigen detection by pattern recognition receptors (including Toll receptors, mannose receptors, dectin1 and dectin 2 receptors as well as C-linked lectin receptors). Because the bulk of their literature citations were from early 2003, IOM was clearly out of date by the time it was published in 2004.

A fundamental concept that links the pathophysiologic mechanisms involved in CIRS-WDB to other systemic inflammatory response syndromes is that inflammatory mediators are blood-borne and not confined within one organ system. These molecules, including cytokines, TGF B-1 and split products of complement activation, are not organ-specific in their effects and will potentially have effects on every tissue receiving a blood supply. Adverse effects from excessive amounts of cytokines, alluded to but not specifically stated by the IOM, will **necessarily be systemic** and not necessarily organ-specific. One cannot have respiratory effects of cytokines, for example, without other

possible health effects, including concomitant cardiovascular, rheumatologic and neurologic effects, among others.

Inclusion bias has been raised by researchers, clinicians, and litigators with regards to publications put forth by the IOM (as will be discussed in review of two non-governmental agency publications, ACOEM (2002) and AAAAI (2006)). By intentionally deleting materials that would not support the consensus opinion, the casual reader of that opinion would likely be unaware that such contradicting data existed. For example, we may reasonably ask if inclusion bias factored into the decision by the IOM to delete a series of papers demonstrating that the illness acquired following exposure to WDB was far greater than simple respiratory problems. Specifically, in the fall of 2003, IOM panel member Harriet Ammann, Ph.D, was given digital copies of five peer-reviewed manuscripts (Campbell, 2003; Campbell, 2004; Crago, 2003; Gray, 2003; and Vojdani, 2003). Each document contained statistically significant, clinically-based data which clearly demonstrated a causal relationship, at a level of reasonable medical and scientific certainty, between exposure to bioaerosols found in WDB and multisystem, multisymptom human illness. Even though these manuscripts had been previously accepted for publication, Dr. Ammann reported that the chairman of the IOM panel refused to distribute the documents, ruling that only materials already in print as of December, 2003 would be considered. Similarly and for unknown reasons, the published work of Shoemaker's group from September 2003 was excluded - it contained a case definition, identification of genetic susceptibility, careful symptom recording, prospective clinical studies, and use of Visual Contrast Sensitivity (VCS), biomarkers and responses to treatment in 156 cases and 111 controls Additionally, we note that neither of the two physicians on the eleven member IOM panel had ever published on diagnosis and treatment of CIRS-WDB. Certainly, given the enormity of the problems posed by CIRS-WDB and potential solutions, inclusion of data and opinions from experienced researchers and treating physicians would greatly enhance the value of recommendations in the development of standards and policy.

As opposed to the IOM panel that was established without input from treating physicians, those within this group have documented the multiple symptom and multisystem aspects of CIRS-WDB. Stated simply, physicians in private or academic practice lacking hands-on knowledge about health effects resulting from exposure to WDB cannot be expected to effectively diagnose and manage this illness. We are aware that the epidemiologic similarity of cases of CIRS-WDB has been published in detail such that others who don't treat the illness cannot understand what elements are involved in CIRS-WDB. We routinely see the need to treat the immunologic basis for this illness after we have corrected the ongoing effects from toxigens.

It is not surprising that both the GAO and WHO reports emphasize immunological contributions to CIRS-WDB illness. The immune responses are well-known to have a basis in individual susceptibility and are often specified by particular haplotypes of HLA DR. We cannot rule out the role other biomarkers, particularly cytochrome P450 markers, glutathione-S-transferases and chitinases, as also contributing

to illness pathogenesis, but the published data are not strong enough to provide clinical certainty at this time.

Notes on health effects from the GAO report:

Page 1: Health effects can arise from immune-mediated and toxic mechanisms.

Page 4: Later reviews concluded that available evidence for additional health effects (rheumatologic, immune diseases) is stronger.

Page 4: Most of the documents warn that certain populations may be more sensitive to mold than others.

Page 33 (table): Most of the federal guidance documents reviewed described populations that may be particularly sensitive to indoor mold though no mention of HLA is included.

Page 11: The one review (AAAAI; see comments below under **Flawed Science** section) that concluded that inhaled toxins were an improbable source of negative health effects **did not address** adverse health effects of mycotoxins that may be caused by immune-mediated mechanisms. Further, the AAAAI paper did not consider fine particulates (i.e. < 1.0 microns) shed by microbial colonies as contributors to indoor bioaerosols, contrary to established literature. We have included a section highlighting the incomplete and inaccurate aspects of the ACOEM and AAAAI reports that remove those reports as sources of reliable information.

Quotes on health effects from the WHO report:

WHO Abstract: The review concludes that the most important effects are an increased prevalence of respiratory symptoms, allergies and asthma as well as perturbation of the immunological system.

Executive summary: "Exposure to microbial contaminants is clinically associated with respiratory symptoms, allergies, asthma and *immunological reactions* (emphasis added)."

Exec Summary, Pg. xiii: "Toxicological evidence obtained in vivo and in vitro supports these findings, showing the occurrence of *diverse inflammatory and toxic responses* after exposure to microorganisms isolated from damp buildings, including their spores, metabolites and components."

Introduction, Pg. 1: "Exposure to microbial contaminants is clinically associated with respiratory symptoms, allergies, asthma and *immunological reactions*."

Introduction Pg. 5: "Mechanisms of injury include exposure to β-glucans, toxins, spores, cell fragments and chemicals followed by immune stimulation, suppression and autoimmunity as well as neurotoxic effects."

Chapter 2, Pg. 16:
"Mycobacteria have also been shown to be common in moisture-damaged buildings, their presence increasing with the degree of fungal damage (Torvinen et al., 2006). Cell wall components of mycobacteria are known to be highly immunogenic, and exposure to mycobacteria may cause inflammatory responses (Huttunen et al, 2000, 2001)."

Chapter 2, pg 17:
"Fungal (1→3)-β-D-glucans are non-allergenic, water-insoluble structural cell wall components of most fungi,...and may account for up to 60% of the dry weight of the cell wall of fungi...(1→3)-β-D-glucans have immunomodulating properties and may affect respiratory health."

Chapter 2, pg 18:
"Mycotoxins, or fungal toxins, are low-relative-molecular-mass biomolecules produced by fungi, some of which are toxic to animals and human beings. Mycotoxins are known to interfere with RNA synthesis and may cause DNA damage. Some fungal species may produce various mycotoxins...Several mycotoxins, e.g. aflatoxin from *Aspergillus flavus* and *Aspergillus parasiticus*, are potent carcinogens. Many mycotoxins are immunotoxic....The mycotoxins that have perhaps received most attention are the trichothecenes, produced by *Stachybotrys chartarum*.....[Mycotoxins] could be present in most samples of materials and settled dust from buildings with current or past damage from damp or water."

Chapter 2, Pg. 19: "These studies demonstrate that mycotoxins are present in the indoor environment and that the levels may be higher in buildings affected by mold and damp."

"*S. chartarum* trichothecene mycotoxins can become airborne in association with both intact conidia and smaller fungal fragments....These studies demonstrate that mycotoxins are present in the indoor environment and that the levels may be higher in buildings affected by mould or damp."

"Several fungi produce volatile metabolites, which are a mixture of compounds....Microbial volatile organic compounds, are often similar to common industrial chemicals. To date, more than 200 of these compounds derived from different fungi have been identified, including various alcohols, aldehydes, ketones, terpenes, esters, aromatic compounds, amines and sulfur-containing compounds.

"Some exposures with adverse health effects associated with damp indoor environments include emissions of volatile organic compounds from damp and mouldy building materials."

Chapter 2, Pg. 32: "Thus, moisture and microbial contamination, not only in the building structure or surfaces, but also in heating, ventilation and air-conditioning systems, has adverse health effects."

Chapter 4, Pg. 63: "Microbiological organisms are considered among the most plausible explanations for the health risks associated with indoor dampness."

Chapter 4, Pg. 78: "Numerous studies have shown that β-glucans have important effects on the human immune system."

Chapter 4, Pg. 80: "Concomitant exposure to endotoxins and curdlan, a (1-3)-β-glucan, was shown to diminish the acute neutrophil response and to augment chronic inflammatory effects (Fogelmark, Sjostrand, Rylander, 1994; Rylander, Fogelmark, 1994). Thus, the effects of inhalation of β-glucans apparently depend on the type of glucans as well as on concomitant exposures."

Chapter 4, Pg. 84:

a. "In two studies, the author found clustering of cases of rheumatic disease in water-damaged buildings (Myllykangas-Luosujarvi et al., 2002; Luosujarvi et al., 2003) and suggested that the symptoms could be attributed to exposure to mold spores. In a later publication (Lange, 2004), the author proposed that endotoxins and other triggers of the innate immune response might play a role."
b. "Rheumatic disease among people exposed in damp buildings and the possible role of endotoxins was also reported by Lorenz et al., (2006)."
c. "This review focuses on the ability of microbial exposures associated with damp buildings to activate the following potential toxicological mechanisms: immunostimulation and allergies, cytotoxicity and immunosuppression, autoimmunity, irritation, neurotoxicity, genotoxity and reproductive toxicity. Novel toxicological data on the role of microbial interactions are also included."
d. "The variety of respiratory symptoms and disease observed in damp and moldy indoor environments suggests that the airways are the primary route of entry for agents."

Chapter 4, Pg. 85:

a. "In damp buildings, people are exposed to constantly changing concentrations of different microbial species, their spores, metabolites and components, and other compounds in indoor air, including chemical emissions from building materials. This complex mixture of exposures inevitably leads to interactions, which affects outcomes in different situations. Furthermore, the effects of microorganisms, microbial substances or dampness-related chemical compounds seen in experimental animals, or cells often result from exposure(s) that are orders of magnitude higher than the average doses that reach the human lungs under normal conditions in indoor air. Nevertheless, the surface doses within the lungs of patients with respiratory conditions can vary a thousand fold, due to uneven particle deposition (Phalen et al., 2006), thus resulting in even larger maximal surface doses in human lungs than in those used in experimental toxicological studies. Moreover, many other factors, such as exercise, can result in larger-than-average doses in the human lung."

b. "Many of the health effects may result from recurrent activation of immune defenses, leading to *exaggerated immune responses* and prolonged production of inflammatory mediators. *Overproduction of these compounds damages the surrounding tissue and may manifest itself as chronic inflammation and inflammation-related diseases (emphasis added).*"

Chapter 4, Pg. 86: "Furthermore, it has been shown in an animal model that immunological status plays an important role in airway inflammation induced by *Stachybotrys chartarum*, enhancing the effects of the mold (Leino et al., 2006). The results imply that sensitized people are more susceptible to exposure to mold than non-atopic (sensitized) people. Different microbial species vary significantly in their immunostimulatory potency in both mouse and human cells in vitro (e.g. Huttunen et al., 2003). Furthermore, it has been clearly demonstrated that different growth conditions and competition between microorganism for the same habitat in vitro change their inflammatory potency, protein expression and toxin production (Ehrlich, 1987)."

"The immunostimulatory activity of Gram-negative bacterial lipopolysaccharide is well-established, but several other bacteria, fungi and isolated mycotoxins associated with damp buildings have been *shown to induce inflammatory responses* in vitro. In line with the findings in vitro, the same microbial species activate acute and sustained inflammation in the lungs of experimental animals."

Chapter 4, Pg 87:

"Fungal spores appear to have toxic effects other than those that cause the inflammatory reaction. Studies of Gram-positive and -negative bacteria (e.g. *Streptomyces californicus*, *Pseudomonas fluorescens*, *Mycobacterium terrae*, *Bacillus cereus*) have shown that the significant difference in cytoxicity among strains is due at least partly to differences in inflammatory activity. Spores and toxins of the fungus *S. chartarum* have been shown to activate the apoptotic pathway....Studies in experimental animals with the same fungal or bacterial species confirm the in vitro findings for cytotoxic effects...as well as lung tissue damage.

"Microbial fragments can...cause autoimmune reactions by molecular mimicry, acting as microbial superantigens or by enhancing the presentation of autoantigens.

"Spores and other particulate material, as well as volatile organic compounds produced by microorganisms, building materials, paints and solvents, are potentially irritating. In epidemiological studies, the prevalence of respiratory and irritative symptoms has been associated with perceived mould odour, possibly indicating the presence of microbial volatile organic compounds."

Chapter 4, Pg. 88: "Such health effects as fatigue, headache, and difficulties in concentration (Johanning et al., 1996; Koskinen et al., 1999b) indicate that microbes or other agents present in damp buildings have neurological effects."

Chapter 4, Pg. 89:

a. "The immunostimulatory properties of the fungal and bacterial strains typically found

in moisture-damaged buildings are synergistically potentiated by microbial interactions during concomitant exposure in vitro (Huttunen et al., 2004)."

b. "Interactions during co-cultivation stimulate these microbes to produce highly toxic compounds, which can damage DNA and provoke genotoxicity (Penttinen et al., 2007). In addition, concomitant exposure in vitro with amoebae potentiates the cytotoxic and inflammatory properties of the microbial spores of *S. californicus* or *Penicillium spinolosum* isolated from damp buildings (Yli-Pirila et al., 2007). These findings point to the importance of considering microbial interactions when investigating the causative agents and mechanisms of the adverse health effects observed in damp buildings."

Chapter 4, Pg. 90: "It is clear, however, that no single mechanism can explain the wide variety of effects associated with dampness and mold. Toxicological studies, by investigating the ability of microbial agents associated with damp buildings to activate certain toxicological mechanisms, provide insight into the multiple biological mechanisms that might underlie the observed associations between health effects and dampness and mold. In vitro and in vivo studies have demonstrated diverse inflammatory, cytotoxic and immunosuppressive responses after exposure to the spores, metabolites and components of microbial species found in damp buildings, lending plausibility to the epidemiological findings."

Chapter 4, Pg. 91:

a. "Various microbial agents with diverse, fluctuating inflammatory and toxic potential are present simultaneously with other airborne compounds, inevitably resulting in interactions in indoor air. Such interactions may lead to unexpected responses, even at low concentrations. Therefore, the detection of individual exposures, such as certain microbial species, toxins or chemical agents, cannot always explain any associated adverse health effects. In the search for causative constituents, toxicological studies should be combined with comprehensive microbiological and chemical analyses of indoor samples."

b. "The synergistic interactions among microbial agents present in damp buildings suggest that the immunotoxic effects of the fungal and bacterial strains typically found can be potentiated during concomitant exposure, leading, for instance, to increased cell death or cytotoxic or inflammatory effects. Such interactions can give rise to unexpected responses, even at low concentrations of microbial (or chemical) agents, so that it is difficult to detect and implicate specific exposures in the causation of damp building-associated adverse health effects. Thus, microbial interactions must be carefully considered when evaluating the possible health effects of exposure in damp buildings."

Chapter 4, Pg. 95: "Building owners are responsible for providing a healthy workplace or living environment that is free of excess moisture and mold, by ensuring proper building construction and maintenance. The occupants are responsible for managing the

use of water, heating, ventilation and appliances in a manner that does not lead to dampness and mold growth."

Notes of health effects from the National Institute of Occupational Safety and Health (NIOSH):

We now know that conditions in damp buildings can lead to respiratory and immunological symptoms in occupants. Two major reviews were published in 2004 and 2009, relating to health effects and damp indoor environments. These were the Institute of Medicine report (IOM, 2004) and the World Health Organization guidelines for dampness and mold (WHO, 2009).

1) The WHO finds, "In many EU countries, 20–30% of households have problems with dampness. Strong evidence indicates that this is a risk to health. In damp conditions, hundreds of species of bacteria and fungi grow indoors and emit spores, cell fragments and chemicals into the air. **Exposure to these contaminants is associated with the *incidence* or worsening of respiratory symptoms**, allergies, asthma and ***immunological reactions***. **Children are particularly susceptible**." World Health Organization, July 2009.)

Major findings were that sufficient evidence existed for associating the presence of mold or other agents in damp buildings with nasal and throat symptoms; cough, wheeze, asthma exacerbations; and hypersensitivity pneumonitis. The IOM committee concluded that limited or suggestive evidence existed for associating exposure to damp indoor environments with shortness of breath, asthma development, and, in otherwise healthy children, lower respiratory disease. The committee noted that immunocompromised individuals are at risk for fungal infections. They concluded that excessive indoor dampness is a public health problem and that prevention or reduction of this condition should be a public health goal (IOM, 2004).

The WHO guidelines covered literature published up to July 2007 on upper respiratory tract symptoms, cough, wheezing, and shortness of breath, asthma exacerbation, asthma development, current asthma, respiratory infections, bronchitis, wheeze, allergic rhinitis, and allergy. It did not cover effects related to skin, eyes, fatigue, nausea, headache, insomnia, mucous membrane irritation, or sick building syndrome (WHO, 2009). In the chapter on health effects, the authors of the WHO guidelines concluded that there is sufficient epidemiological evidence of an association between indoor dampness-related factors and asthma development, asthma exacerbation, current asthma, respiratory infections, upper respiratory tract symptoms, cough, wheeze and shortness of breath. Updated findings comparing the WHO guidelines and IOM report conclude that there is now sufficient evidence of an association between indoor dampness-related agents and the development of asthma, current asthma, shortness of breath, and respiratory infections. There is now limited or suggestive evidence for bronchitis and allergic rhinitis being associated with dampness-related exposures. There is clinical evidence that exposure to mold and other dampness-related microbial agents increase the risk for

hypersensitivity pneumonitis, chronic rhino-sinusitis, and allergic fungal sinusitis. Importantly, the WHO guidelines note that although atopy and allergy increase susceptibility to dampness-related health effects, such health effects are also found in non-atopic building occupants. Therefore, dampness is a strong, consistent indicator of risk for adverse health effects on the human immune system. The WHO guidelines relevant to occupational environments are as follows:

- Persistent dampness and microbial growth on interior surfaces and in building structures should be avoided or minimized, as they may lead to adverse health effects.

- Indicators of dampness and microbial growth include the presence of condensation on surfaces or in structures, visible mold, perceived moldy odor and a history of water damage, leakage or penetration. Thorough inspection and, if necessary, appropriate measurements, can be used to confirm indoor moisture and microbial growth.

- As the relationships between dampness, microbial exposure and health effects cannot be quantified precisely, no quantitative health-based guideline values or thresholds can be recommended such that that dampness and mold-related problems will be prevented. When they occur, they should be remediated because they increase the risk of hazardous exposure to microbes and chemicals.

- Well-designed, well-constructed, well-maintained building envelopes are critical to the prevention and control of excess moisture and microbial growth, as they prevent thermal bridges and the entry of liquid or vapor-phase water. Management of moisture requires proper control of temperatures and ventilation to avoid excess humidity, condensation on surfaces and excess moisture in materials. Ventilation should be distributed effectively throughout spaces. And stagnant air zones should be avoided.

- Building owners are responsible for providing a healthy workplace or living environment free of excess moisture and mold, by ensuring proper building construction and maintenance. The occupants are responsible for managing the use of water, heating, ventilation and appliances in a manner that does not lead to dampness and mold growth.

We do not recommend routine air sampling for mold as a sole indicator of potential cause of ill health because air concentrations of molds or spores alone cannot be interpreted with regard to health risk in most instances.

Instead we encourage detection by thorough visual inspections and/or detection via musty odors. These methods cost nothing and do correlate with health risk in buildings with indoor environmental complaints. Consultants sometimes identify sources of dampness with moisture meters and infrared cameras.

Once sources of moisture have been identified, we recommend that mold and moisture-damaged materials be cleaned or removed with appropriate containment to minimize exposure to building occupants and that necessary repairs be made to prevent further water entry into the building.

ANSI-IICRC S520-2006 *Standard and Reference Guide for Professional Mold Remediation*.
Fundamental principles: Identify and stop the moisture source.
Remove affected materials without cross-contamination of surrounding areas.
Remove settled spores from surfaces.

After remediation, employees and management often wish to know if the building is "safe." Building consultants often recommend and perform "clearance" air sampling after work has been completed in an attempt to demonstrate that the building is safe for occupants. *However, there is no scientific basis for the use of air sampling for this purpose. Once remediation is completed (moldy and damaged materials removed; musty odors no longer evident), the best evidence that the building is safe may be that employees no longer experience building-related symptoms (emphasis added).*

ACGIH *Bioaerosols* 8.6.3:
"*Whenever possible, reaching a decision that cleanup or abatement efforts have succeeded should include an assessment of the acceptability of the environment by the workers who will occupy the space (emphasis added)."*

ACGIH *Bioaerosols* 15.5:
"*The ultimate criterion for the adequacy of abatement efforts for treating biological contamination is the ability of people to occupy or re-occupy the space without health complaints or physical discomfort (emphasis added)."*

American Conference of Governmental Industrial Hygienists (ACGIH) *Bioaerosols*, 1999, Ed. Janet M. Macher, Sd.D. M.P.H.

The U.S Environmental Protection Agency publication *Mold Remediation for Schools and Commercial Buildings*, page 26, point 5:
"People should be able to occupy or re-occupy the space without health complaints or physical symptoms."

Physiologic parameters and neurotoxicological testing, such as visual contrast sensitivity (VCS) and the Kilburn Neurotest Battery (KNB), will add to diagnostic utility in screening for illness once appearance of new symptoms provides impetus for further evaluation.

Note: While NIOSH doesn't comment above about residential exposures, the same language applies to residential construction with water intrusion and the occupants.

Biocontaminants in WDB.

Jack D. Thrasher, PhD

Introduction: Increased moisture in homes and buildings leads to the amplification of growth of molds, bacteria and their by-products (1-15). The resultant contamination produces complex consequences to human and animal health and will be briefly reviewed here. (Note: This material is published in greater detail by Thrasher JD and Crawley S, Toxicolo Indust Health 2009; 25:584-615.)

1. Signal Molds: Certain species of molds grow better indoors versus outdoors. Therefore, the species of molds indoors versus outdoors must be compared, understanding that a single measure of total counts outdoors versus indoors has no meaning. The species of concern are: *Aspergillus flavus, versicolor, fumigatus, penicillioides, terreus, ochraceus, niger*; *Penicillium brevicompactum, citrinum, chrysogenum, decumbens*; *Stachybotrys chartarum* (Chemotypes A and M); *Chaetomium spp*, *Epicoccum spp*, *Cladosporium cladosporioides* (types 1 & 2), *herbarum; Fusarium spp*; *Ulocladium botrytis; Trichoderma viride; Eurotium amstelodami* (1-8, 16, 17, 20). In addition, multiple sampling methods (e.g. PCR-DNA, culture, dust) are needed to fully characterize the mold growth in WDB (16, 18, and 19).

2. Mycotoxins: Mycotoxins are produced by several different species of molds. The mycotoxins detected in dust, carpeting, building materials and air of damp indoor spaces includes: Aflatoxin B1, sterigmatocystin, trichodermol, trichodermin, verrucarin; macrocyclic trichothecenes, and gliotoxin (6, 21-26). Macrocyclic trichothecene concentration ranged from 0.01-1.3 ng/m^3 in one report of environmental samples (6) and was also identified qualitatively in the sera of affected individuals (27). Airborne concentrations of Satratoxin G and H were detected at 0.25 ng and 0.43 ng per m^3 corresponding to a dose of 2.0 ng SG+SH per 8 hour day at respiratory volume of 6 liters, at rest (21). Harmful concentrations of mycotoxins in building materials in WDB have been reported (26) as well as in dust samples: gliotoxin (0.45-1.13 pg/mg); sterigmatocystin (4.9 – 150,000 pg/mg); trichodermol (0.9-8700 pg/mg) and verracarol (8.8-17,000 pg/mg); aflatoxin B_1 (32 & 14500 pg/$cm^{2)}$; sterigmatocystin (3.6-10900 pg/$cm^{2)}$; trichodermol (6.5- 170 pg/$cm^{2)}$; verracarol (15-3400 pg/$cm^{2)}$; and gliotoxin (400 pg/$cm^{2)}$. Thus, mycotoxins are present at unhealthy levels in building materials, dust samples and air samples of WDB.

S. chartarum consists of two distinct genotypes. One produces macrocyclic trichothecenes (Type M) and the other atranones (Type A) (for review, see 81). Exposure to the atranones induces inflammatory responses (increased macrophages and neutrophils and the production of pro-inflammatory cytokines, including MIP-2, TBF-α, and IL-6 in the broncho-alveolar lavage (BAL) of mice.

S. chartarum also produces up to 40 different spirocyclic drimanes (for review see 81). They have a wide spectrum of biological activities: inhibition of proteolytic enzymes; disruption of the complement system; inhibition of TNF-α release; endothelin

receptor antagonism and stimulation of plasminogen, fibrinolysis, thrombolysis and cytotoxic and neurotoxic effects.

3. Bacteria: Gram negative and positive bacteria have been identified in a variety of indoor settings, day care centers, schools, hospitals and WDB. These bacteria are potent inducers of pro-inflammatory cytokines in vitro and in vivo (28-33).

A. Gram Negative Bacteria: This group includes *E. coli*, *Pseudomonas, Enterobacter, Agrobacteria*, *Caulobacter, Stenophomonas, Chryseomonas* and *Acinetobacter*. These are potential human pathogens and release endotoxins (lipopolysaccharides, LPS) (34). Other bacteria, which require additional studies for clear delineation of their role in illness causation, present in indoor environments include the following families: *Sphingomonadaceae, Oxalobacteraceae, Comamonadaceae, Neisseriaceae and Rhizobiaceae* (35).

B. Gram Positive Bacteria: Species of *Streptococcus, Staphylococcus* (particularly multiple antibiotic resistant coagulase negative species), *Bacillus* and *Actinobacteria* (actinomycetes) have been identified in WDB. Of the *Actinobacteria,* several species of *Streptomyces, Actinomyces*, *Nocardia, Propionibacteria* and *Cornynebacteria* have been reported to be found to be in WDB and also to cause illnesses. Pathogenic *Mycobacteria* are also reported to be in the complex mixture of microbes found inside WDB (9-15, 35-41). The *Actinobacteria* are potential human pathogens. In addition, several genera have been associated with lung disease (hypersensitivity pneumonitis [HP] and sarcoidosis), though causation is not confirmed in sarcoid. Several species of *Mycobacterium* can cause Mycobacterium avium complex (MAC); in addition *Mycobacteria ulcerans* and related mycolactone-formers make toxins (86, 87). Mycetoma may be present with infections from *Streptomyces, Actinomyces, Mycobacterium* and *Nocardia*.

Recent research has associated *Mycobacterium* with sarcoidosis. The evidence includes: PCR DNA testing for 16Dr-RNA (2002); a meta-analysis of molecular studies on the role of mycobacteria in sarcoidosis; and induction of interferon-γ by peripheral blood mononuclear cells from sarcoidosis patients and recognition of the antigens by TLR-2 receptors. (82-85). Thus, we must be aware of the potential role of all *Actinobacteria* in WDB illness.

4. MVOCs: Mold and bacteria produces microbial volatile organic compounds (MVOCs) that admix with VOCs released by consumer goods. The MVOCs include a variety of alcohols, organo-disulfides, limonene, ketones, aldehydes, amines, furans, ammonia, terpenes and geosmin. Note that geosmin produces the odor of wet soil. These can be irritating to the mucous membranes of the eyes, nose, throat and lungs as well as adding to the overall toxicity of indoor air of WDB (42-47).

5. 1-3—Beta-D-Glucans (beta glucans, glucans): Glucans are polysaccharides of the cell wall of molds. Detection in indoor environments can be used as a marker of mold contamination (48-56). The glucans are irritants to the mucous membranes of the eyes, throat, upper and lower respiratory tract. They cause variability in air flow in children,

inflammatory reactions of the airways and eosinophilia. In addition they are immunogenic. The inflammation involves TLR 2, TLR4, MYD88, dectin-1 and dectin-2 receptors (57-59).

6. Galactomannans (EPS): EPS are cell wall polysaccharides of molds. They are highly branched (1-2, 1-5 & 1-6 linkages). Their presence in indoor environments is indicative of mold contamination. They are irritants to the mucous membranes of the eyes, throat and upper and lower airways (60-62).

7. Endotoxins, also called lipopolysaccharides: Lipopolysaccharides (LPS) are cell wall components of Gram negative bacteria. They are shed into the environment of WDB upon death of the bacteria. LPS cause an inflammatory response via cell marker CD14, TLR-4 and long pentraxin (PTX3) signaling pathways, releasing inflammatory cytokines (e.g. TNF-α). LPS aggravate pre-existing lung disease (asthma, HP), can cause inflammation of the lungs and are synergistic with mycotoxins *in vivo* and *in vitro* (63-72). The CD14 locus appears to have a central role in the sensitivity to LPS.

8. Hemolysins and various proteins: Molds and bacteria release protein-based enzymes (lipases, chitinases, amylases, proteinases, etc.) into the substrates upon which they are growing. These proteins are antigenic. Hemolysins and siderophores are produced by eleven species of *Aspergillus*, ten species of *Penicillium*, two species of *Ulocladium*, *Paecilomyces variotil, Memnoniella echinata, Scopulariopsis brevicaulis*, *Trichoderma viride* and *longibrachiatum* and *S. chartarum*. Thus, several different genera of molds are capable of adding to hemolysis and upper and lower respiratory bleeding (73-75).

9. Particulates: Particulates are shed from microbial (molds and bacteria) colonies as a result of normal human activity. Older colonies shed more readily than younger colonies. The particulates are the source of the majority of contaminants listed above. They have been classified into three ranges: <1 micron (**fine particulates**); 1.1 to 2.5 microns; and ≥2.5 microns. These ranges are based upon the detection of 1-3-β-D-glucan (cell walls of mold) in indoor environments and, therefore, do not necessarily represent all **fine particulates**, e.g. endotoxins, shed by microbial colonies. Nonetheless, the fine particulates are estimated to be up to 500 times greater in concentration than are mold spores. The contaminants absorbed on fine particulates enter the human body by translocation: (1) across the alveolar membrane into the systemic circulation and (2) transport into the olfactory bulbs via the olfactory mucosa and tract (55, 56, 76-78).

Comments Regarding Indoor Microbial Sampling and Culturing

A single indoor air sample, or even a small number of samples, for viable and nonviable spores, does not represent the actual indoor contamination resulting from microbial growth in WDB because: (1) the sampling is only a snapshot in time of the environment; (2) bacteria and other micro-organisms and their by-products are not sampled; (3) levels and density of airborne mold spores fluctuate during the day; (4) fine particulates and

their contaminants are not determined; (5) much, if not all, of the microbial growth is often hidden from view (e.g. wall cavities, attics, under carpeting, behind base boards); (6) one-time sampling will not reflect changes in air flow over time, and the resultant burden of bioaerosols if (i) there is a basement or crawlspace, (ii) if there is HVAC distributing air, or (iii) if there is ongoing activity in the room being sampled, such as presence of people and pets, use of fans, opening or closing windows. Therefore, multiple sampling and analytical methods should be considered including bulk sampling. None of these sampling techniques measure exposure, only presence. Exposure is a measure of presence (in the breathing zone or where skin contact or ingestion may occur) over time in a structure with common activities occurring.

1. Mold Testing by DNA Analysis (PCR): PCR analysis of dust or bulk samples for mold accurately identifies as many as 160 mold species, but not their quantities. Several samples should be collected because the mold species will vary based upon the substrate sampled. Sampling with cloths, such as Swiffer cloths, can be used if vacuuming is not available. Such measures will also not give quantitative assessments of total burden of fungi, just a highly accurate determination of species collected (see 88, 89). To date the only PCR testing readily available commercially assesses fungal DNA and not DNA of bacteria, actinomycetes or mycobacteria.

An application of PCR analysis (Environmental Relative Moldiness Index, ERMI) was released by the US EPA and licensed to accredited laboratories in 2006. EPA research developed a DNA library limited to a combination of 36 species they identified as representative of WDB in published work. The results of the PCR analysis are used to calculate an arbitrary single building index number identified as ERMI. This index has been used by medical personnel to correlate with human health indices obtained as part of their evaluation of risk of re-exposure. But there is no basis as yet, however, to conclude that the ERMI value alone (i) is reliable as an independent parameter of risk for adverse health effects of occupancy and (ii) will be one that can be used as an independent assessment of building health without confirmation by additional means including on-site assessment made by credentialed experts in indoor hygiene. We note that a recent EPA fact sheet on ERMI (www.epa.gov) announced that there may be little or no need for any routine measurement of indoor indices of building health in homes, consistent with the earlier opinion of Health Canada (Minister of Health. Residential indoor air quality guideline for moulds; 2007-03-31 Canada Health Gazette Part I 141(13)) that microbial measurements cannot be used to assess risk to the health of building occupants. However, the EPA also states in that same release that, “Testing may also be useful to help identify or characterize the magnitude of specific mold problems in some indoor environments.” Environments that host sickened people may logically be included in such an assessment of mold problems. Further research is requested and is underway.

Experts involved with building health assessment will provide guidance for physicians as further research streamlines the search for newer tools that provide a broader and more accurate assessment of building health.

The ERMI analysis can still be used for the identification of the 36 species. The ERMI index number is one of several tools currently available to a treating physician in a different diagnostic process. No single diagnostic process alone suffices for complete human health assessment, but each is a separate spoke of the entire diagnostic wheel. The correlation of ERMI or other site data with human health effects can only be made by a physician with access to **both** the human health data and the building health data. Here, the role of the treating physician emerges as participant with a team of professionals involved with care and management of a WDB and the occupants therein.

2. Mold Testing by Microscopy: Quantification of mold spores is possible by analyzing samples collected from surfaces, in dust, bulk, or air by a trained microscopist. Numbers include the total fungal spores collected, whether viable or not, further detailed by genus. Unlike ERMI and PCR testing, almost no species can be accurately identified, only genera. Some genera cannot be differentiated because distinguishing characteristics occur in the growth structures and not in the spore itself. The primary example is *Penicillium* and *Aspergillus*, which is reported as Pen/Asp-type. Microscopy results, especially for air samples, will often have a debris rating. This number indicates an increased error rate in counting spores because of all the other ubiquitous particulate collected obscure the visibility of the spores. Although some hyphal fragments can be identified, the count is not reliable and no further identification can be made, even at the genus level. Analysis by microscopy can be performed within minutes after arrival at the lab.

3. Mold Testing by Culturing: Quantification of mold spores which germinate and form a colony is possible by analyzing samples collected from surfaces, in dust, bulk, or air by a trained microscopist. Numbers include the total fungal spores collected which were viable and which formed growth colonies on the specific agar in the culture plate. Unlike microscopy which ideally will reveal all spores collected, culturing reveals only those spores that can utilize the specific agar in the culturing plate under the specific laboratory conditions and out-compete other genera. Culturing, however, can accurately identify viable species because growth structures are present revealing the morphology of each organism. Results of culturing are available only after the spores have time to germinate and form colonies. The time range is from a few days to as long as a couple of weeks. Detailed speciation requires additional time, occasionally as long as a month or more. Thorough identification of all organisms present will require PCR identification of non-viable species.

Assessment of non-viable versus viable spores may be misleading in that the vast majority of the source of total inflammatory burden posed by exposure to the interior environment of WDB resides in fragments of spores and hyphae, with such fragments in the range of 0.45 to 0.60 microns being of singular importance. Assessment of spores will not indicate fragments and as such are not representative of the total inflammatory burden.

Given the limitations of ERMI and spore counting, the field of indoor air sampling is involved actively in research to develop a more universal, cost-effective building index

assessment. Until the time that new research creates a better test, ERMI, other PCR methods and spore counting will continue to be used routinely.

4. Mycotoxins: Mycotoxin contamination can be determined if deemed necessary. Dust and bulk samples can be tested by the following labs: Real Time Laboratories, US Bureau Veritas, Mycometrics and EMSL. Such sampling is rarely required as individual susceptibility and host response factors to levels of exposure below levels of detection are commonly seen. In addition, the potential for sampling error and high cost are prohibitive for the necessary multiple analyses. Bulk sampling greatly reduces errors associated with air sampling.

5. Culture Temperatures: Certain molds and bacteria have optimum growth temperatures.

A. Molds: Different molds require different growth conditions. For example, the optimal temperature for *Aspergillus flavus* and is out-competed by the faster growing *Penicillium* species.

B. Bacteria: Media should be used that will culture *Actinobacteria (also called Actinomycetes)*. Also, blood agar should be used to culture for Gram negative bacteria. Culture temperatures should be between 55 and 37^{o} C. Thermophilic *Actinobacteria* can be expected and have been shown to cause lung disease. *Actinobacteria* are slow-growing and cultures should be maintained for several days.

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Biocontaminants Causing Human Illness

Notes from GAO:

The multiple agents (including toxins and inflammagens) found inside WDB could individually cause similar inflammatory responses. Trying to distinguish which agent is responsible for a specific inflammatory response is not possible. As discussed previously so-called, "specific causation" simply doesn't exist. However, there is a significant data base demonstrating that even if not measured specifically, the multiple inflammagens and toxigens that can cause illness ***will be found*** in WDB.

The following comments are extracted from the GAO and WHO reports followed by discussions from specific academic papers.

GAO:

Letter to Senator Kennedy; Pg 1:

Several components of mold may cause disease.

Fragments may cause adverse health effects.

Certain components of cell walls may cause adverse health effects.

Page 4:

There is difficulty in determining which of several disease-causing agents in damp indoor environments may be responsible for adverse health effects.

There is a need for research to determine the health effects of long-term exposure to the toxins that some molds can produce.

Two key factors: page 16.

A wide variety of potential disease-causing agents are likely to be present in damp indoor environment, which makes it difficult to link health effects with specific agents.

Potential disease-causing agents, including many species of mold and other biological agents, such as bacteria, are likely to be present in damp indoor environments.

Several different components or products of mold, such as mycotoxins, may function as disease-causing agents in indoor environments.

Appendix I: Objectives, scope and methodology.

GAO searched 19 different terms on PubMed: mold, exposure, health, indoor, glucan, microbial VOCs, mycotoxins, ergosterol, hemolysins, fungal extracellular polysaccharides, fungal/hyphal fragments, allergens, *Stachybotrys,* acute idiopathic pulmonary hemorrhage, acute pulmonary hemorrhage and infants and hemosiderosis.

Quotes from WHO (some cited earlier):

WHO Abstract:

"Indoor air pollution is caused by hundreds of species of bacteria and fungi, in particular filamentous fungi (mould), growing indoors when sufficient moisture is available."

WHO Executive Summary:

Pg. xi-xii:

"There is strong evidence regarding the hazards posed by several biological agents that pollute indoor air; however, the WHO working group convened in October 2006 concluded that the individual species of microbes and other biological agents that are responsible for health effects cannot be identified. This is due to the fact that people are often exposed to multiple agents simultaneously, to complexities in accurately estimating exposure and to the large numbers of symptoms and health outcomes due to exposure.

"The presence of many biological agents in the indoor environment is due to dampness and inadequate ventilation. Excess moisture on almost all indoor materials leads to growth of microbes, such as mould, fungi and bacteria, which subsequently emit spores, cells fragments and volatile organic compounds into indoor air.

WHO Exec Summary, Pg. xiii:

"Toxicological evidence obtained in vivo and in vitro supports these findings, showing the occurrence of diverse inflammatory and toxic responses after exposure to microorganisms isolated from damp buildings, including their spores, metabolites and components."

WHO Exec Summary, Pg. xiv:

"The amount of water on or in materials is the most important trigger of the growth of microorganisms, including fungi, actinomycetes and other bacteria.

"Health hazards result from a complex chain of events that link penetration of water indoors, excessive moisture to biological growth, physical and chemical degradation, and emission of hazardous biological and chemical agents." Executive Summary, page XIII.

"Microbial growth may result in greater numbers of spores, cell fragments, allergens, mycotoxins, endotoxins, β-glucans and volatile organic compounds in indoor air. The causative agents of adverse health effects have not been identified conclusively, but an excess level of any of these agents in the indoor environment is a potential health hazard."

"Toxicological evidence obtained in vivo and in vitro supports these findings, showing the occurrence of diverse inflammatory and toxic responses after exposure to microorganisms isolated from damp buildings, including their spores, metabolites and components."

WHO Exec Summary, Pg. xv:

"As the relations between dampness, microbial exposure and health effects cannot be quantified precisely, no quantitative health-based guideline values or thresholds can be recommended for acceptable levels of contamination with microorganisms. Instead, it is recommended that dampness and mould-related problems be prevented."

WHO Introduction, Pg. 5:

"Mechanisms of injury include exposure to β-glucans, toxins, spores, cell fragments and chemicals followed by immune stimulation, suppression and autoimmunity as well as neurotoxic effects."

WHO Chapter 2, Pg. 9:

"Indoor environments contain a complex mixture of live (viable) and dead (non-viable) microorganisms, fragments thereof, toxins, allergens, volatile microbial organic compounds and other chemicals. The indoor concentrations of some of these organisms and agents are known or suspected to be elevated in damp indoor environments and may affect the health of people living or working there."

WHO Chapter 2, Pg. 15:

"Many fungi and some yeast replicate by producing numerous spores that are well adapted to airborne dispersal....They can stay airborne for long periods and may deposit in the respiratory system, some smaller spores reaching the alveoli. Fungi can release even smaller fungal fragments, which are derived from broken or fractured spores and hyphae and can be categorized into submicron particles...Even more fungal fragments than spores may be deposited in the respiratory tract; like spores, they are known to contain allergens and mycotoxins."

WHO Chapter 2, Pg. 16:

"Mycobacteria have also been shown to be common in moisture-damaged buildings, their presence increasing with the degree of fungal damage (Torvinen et al., 2006). Cell wall

components of mycobacteria are known to be highly immunogenic, and exposure to mycobacteria may cause inflammatory responses (Huttunen et al, 2000, 2001)."
"In the environment, airborne endotoxins are usually associated with dust particles or aqueous aerosols.

"Indoor fungal fragments are not commonly measured in field studies, but a study with an aerosolization chamber showed that submicron fungal fragments from culture plates and mould-contaminated ceiling tiles aerosolized simultaneously with spores but at substantially higher concentrations (320 - 524 times higher). This suggests that indoor exposure to fungal fragments is at least as important as exposure to fungal spores."

WHO Chapter 2, pg 17:

"Fungal (1→3)-β-D-glucans. (1→3)-β-D-glucans are non-allergenic, water-insoluble structural cell-wall components of most fungi, and may account for up to 60% of the dry weight of the cell wall of fungi.... (1→3)-β-D-glucans have immunomodulating properties and may affect respiratory health."

WHO chapter 2, pg 18:

"Mycotoxins, or fungal toxins, are low-relative-molecular-mass biomolecules produced by fungi, some of which are toxic to animals and human beings. Mycotoxins are known to interfere with RNA synthesis and may cause DNA damage. Some fungal species may produce various mycotoxins...Several mycotoxins, e.g. aflatoxin from *Aspergillus flavus* and *Aspergillus parasiticus*, are potent carcinogens. Many mycotoxins are immunotoxic....The mycotoxins that have perhaps received most attention are the trichothecenes, produced by *Stachybotrys chartarum*.....[Mycotoxins] could be present in most samples of materials and settled dust from buildings with current or past damage from damp or water."

WHO Chapter 2, Pg. 19:

"These studies demonstrate that mycotoxins are present in the indoor environment and that the levels may be higher in buildings affected by mold and damp.

"*S. chartarum* trichothecene mycotoxins can become airborne in association with both intact conidia and smaller fungal fragments....These studies demonstrate that mycotoxins are present in the indoor environment and that the levels may be higher in buildings affected by mould or damp."

"Several fungi produce volatile metabolites, which are a mixture of compounds....Microbial volatile organic compounds, are often similar to common industrial chemicals. To date, more than 200 of these compounds derived from different fungi have been identified, including various alcohols, aldehydes, ketones, terpenes, esters, aromatic compounds, amines and sulfur-containing compounds."

"Some exposures with adverse health effects associated with damp indoor

environments include emissions of volatile organic compounds from damp and mouldy building materials."

WHO Chapter 4, Pg. 63:

"Microbiological organisms are considered among the most plausible explanations for the health risks associated with indoor dampness."

WHO Chapter 5, Pg. 75:

"The exposures that cause dampness-related illness have not yet been determined. A study of an association between health effects and the concentration of a specific microorganism or microbial compound is in fact testing a hypothesis. In the studies in our review, such hypothetical causal exposures included all culturable fungi, all fungal spores, species-specific spores, all fungal biomass (ergosterol) (Robine et al., 2005), the total mass of specific organism (*Aspergillus* and *Penicillium* extracellular polysaccharides) and specific toxic compounds (endotoxins, β-glucans)."

WHO Chapter 4, Pg. 78:

"Numerous studies have shown that β-glucans have important effects on the human immune system."

WHO Chapter 4, Pg. 80:

"Concomitant exposure to endotoxins and curdlan, a (1-3)-β-glucan, was shown to diminish the acute neutrophil response but to augment chronic inflammatory effects (Fogelmark, Sjostrand, Rylander, 1994; Rylander, Fogelmark, 1994). Thus, the effects of inhalation of β-glucans apparently depend on the type of glucans as well as on concomitant exposures."

WHO Chapter 4, Pg. 85:

"In damp buildings, people are exposed to constantly changing concentrations of different microbial species, their spores, metabolites and components, and other compounds in indoor air, including chemical emissions from building materials. This complex mixture of exposures inevitably leads to interactions, which affects outcomes in different situations. Furthermore, the effects of microorganisms, microbial substances or dampness-related chemical compounds seen in experimental animals or cells often result from exposure that are orders of magnitude higher than the average doses that reach the human lungs under normal conditions in indoor air. Nevertheless, the surface doses within the lungs of patients with respiratory conditions can vary a thousand fold, due to uneven particle deposition (Phalen et al., 2006), thus resulting in even larger maximal surface doses in human lungs than in those used in experimental toxicological studies. Moreover, many other factors, such as exercise, can result in larger-than-average doses in the human lung."

"Many of the health effects may result from recurrent activation of immune defense, leading to exaggerated immune responses and prolonged production of inflammatory mediators. Overproduction of these compounds damages the surrounding tissue and may manifest itself as chronic inflammation and inflammation-related diseases."

WHO Chapter 4, Pg. 86:

"Furthermore, it has been shown in an animal model that immunological status plays an important role in airway inflammation induced by *Stachybotrys chartarum*, enhancing the effects of the mold (Leino et al., 2006). The results imply that sensitized people are more susceptible to exposure to mold than non-atopic people. Different microbial species differ significantly in their immunostimulatory potency in both mouse and human cells in vitro (e.g. Huttunen et al., 2003). Furthermore, it has been clearly demonstrated that different growth conditions and competition between microorganism for the same habitat in vitro change their inflammatory potency, protein expression and toxin production (Ehrlich, 1987)."

"The immunostimulatory activity of Gram-negative bacterial lipopolysaccharide is well established, but several other bacteria, fungi and isolated mycotoxins associated with damp buildings have been shown to induce inflammatory responses in vitro. In line with the findings in vitro, the same microbial species activate acute and sustained inflammation in the lungs of experimental animals."

WHO chapter 4, Pg 87:

"Fungal spores appear to have toxic effects other than those that cause the inflammatory reaction. Studies of Gram-positive and -negative bacteria (e.g. *Streptomyces californicus*, *Pseudomonas fluorescens*, *Mycobacterium terrae*, *Bacillus cereus*) have shown that the significant difference in cytoxicity among strains is due at least partly to differences in inflammatory activity. Spores and toxins of the fungus *S. chartarum* have been shown to activate the apoptotic pathway....Studies in experimental animals with the same fungal or bacterial species confirm the in vitro findings for cytotoxic effects...as well as lung tissue damage."

"Microbial fragments can...cause autoimmune reactions by molecular mimicry, acting as microbial superantigens or by enhancing the presentation of autoantigens."

"Spores and other particulate material, as well as volatile organic compounds produced by microorganisms, building materials, paints and solvents, are potentially irritating. In epidemiological studies, the prevalence of respiratory and irritative symptoms has been associated with perceived mould odour, possible indicating the presence of microbial volatile organic compounds."

WHO Chapter 4, Pg. 88:

"Such health effects as fatigue, headache and difficulties in concentration (Johanning et al., 1996; Koskinen et al., 1999b) indicate that microbes or other agents present in damp buildings have neurological effects."

WHO Chapter 4, Pg. 89:

"The immunostimulatory properties of the fungal and bacterial strains typically found in moisture-damaged buildings are synergistically potentiated by microbial interactions during concomitant exposure in vitro (Huttunen et al., 2004).

"Interactions during co-cultivation stimulate these microbes to produce highly toxic compounds, which can damage DNA and provoke genotoxicity (Penttinen et al., 2007). In addition, concomitant exposure in vitro with amoebae potentiates the cytotoxic and inflammatory properties of the microbial spores of *S. californicus* or *Penicillium spinolosum* isolated from damp buildings (Yli-Pirila et al., 2007). These findings point to the importance of considering microbial interactions when investigating the causative agents and mechanisms of the adverse health effects observed in damp buildings."

WHO Chapter 4, Pg. 90:

"It is clear, however, that no single mechanism can explain the wide variety of effects associated with dampness and mold. Toxicological studies, by investigating the ability of microbial agents associated with damp buildings to activate certain toxicological mechanisms, provide insight into the multiple biological mechanisms that might underlie the observed associations between health effects and dampness and mold. In vitro and in vivo studies have demonstrated diverse inflammatory, cytotoxic and immunosuppressive responses after exposure to the spores, metabolites and components of microbial species found in damp buildings, lending plausibility to the epidemiological findings."

WHO Chapter 4, Pg. 91:

"Various microbial agents with diverse, fluctuating inflammatory and toxic potential are present simultaneously with other airborne compounds, inevitably resulting in interactions in indoor air. Such interactions may lead to unexpected responses, even at low concentrations. Therefore, the detection of individual exposures, such as certain microbial species, toxins or chemical agents, cannot always explain any associated adverse health effects. In the search for causative constituents, toxicological studies should be combined with comprehensive microbiological and chemical analyses of indoor samples."

Literature from non-governmental agencies:

The synergistic interactions among microbial agents present in damp buildings suggest that the immunotoxic effects of the fungal and bacterial strains typically found can be potentiated during concomitant exposure, leading, for instance, to increased cell death or cytotoxic or inflammatory effects. Such interactions can give rise to unexpected responses, even at low concentrations of microbial (or chemical) agents, so that it is difficult to detect and implicate specific exposures in the causation of damp building-associated adverse health effects. Thus, microbial interactions must be carefully considered when evaluating the possible health effects of exposure in damp buildings.

The following is an **Annotated List of References** confirming presence of mycotoxins and other sources of inflammation found in WDB, *if the testing is done*:

1. American Industrial Hygiene Association (AIHA) *Recognition, Evaluation, and Control of Indoor Mold.* 2008. Editors Bradley Prezant, MSPH, CIH, CPE; Donald M. Weekes, CIH, CSP; J. David Miller, PhD.

Chapter 1, Section 1.3.5:
Indoor exposures are a complex mixture of molds, bacteria, fragments of both types of organisms; their multiple toxic products; and biologically derived small particles, gases and other air pollutants. Effects, depending on the susceptibility of the exposed occupants and their degree of exposure, can be combinations of allergic response, inflammation and its consequences, and other toxic responses. This complex exposure and effect picture is not addressed by risk assessment focused on spores or individual toxins.

2. Skaug MA, Eduard W, Stormer FC. Ochratoxin A in airborne dust and fungal conidia. Mycopathologia 2001; 151(2): 93-98.

This paper from 2001 looks at the question of whether or not mycotoxins are present in fungal fragments in airborne dust in a water-damaged building. They looked at ochratoxin, and were able to reproducibly identify it in picogram quantities from extracts of fungal conidia. The authors concluded that airborne dust and fungal conidia can be sources of ochratoxin.

3. Bloom E, Bal K, Nyman E, Must A, Larsson L. Mass spectrometry-based strategy for direct detection and quantification of some mycotoxins produced by *Stachybotrys* and *Aspergillus* spp. in indoor environments. Applied and Environmental Microbiology 2007; 73(13): 4211-4217.

This is another paper looking at whether or not mycotoxins are found in water-damaged buildings. The authors use mass spectroscopy to confirm that mycotoxins produced by *Aspergillus* and *Stachybotrys* are found in buildings with ongoing dampness and a history of water damage. The direct detection of highly toxic sterigmatocystin and macrocyclic trichothecene mycotoxins in indoor environments is important to their potential health impacts. Direct detection of mycotoxins in 45 of 62 building material samples, three of

eight sets of dust samples, and five of eight cultures of airborne dust samples confirm the benefit of high performance liquid chromatography-tandem mass spectrometry.

4. Gottschalk C, Bauer J, Meyer K. Detection of satratoxin G and H in indoor air from a water-damaged building. Mycopathologia 2008; 166: 1103-7.

This 2008 study used a 0.8 micron filter to trap particulates from the indoor air of a WDB. Satratoxin G and H were found to be airborne at concentrations of 0.25 and 0.43 ng/cubic meter. These finding support those of Brasel, et al, 2005.

5. Engelhart S, Loock A, Skutlarek D, Sagunski H, Lommel A, Farber H, Exner M. Occurrence of toxigenic *Aspergillus versicolor* isolates and sterigmatocystin in carpet dust from damp indoor environments. Applied and Environmental Microbiology 2002; 68(8): 3886-3890.

This is a paper published in August 2002, well before the ACOEM statement was released, that confirms the presence of mycotoxins in carpet tests in areas of homes with water damage. The authors conclude that the *Aspergillus versicolor* that were isolated from carpet dust were able to produce sterigmatocystin and that this toxin itself was readily isolated from carpet dust. 98% of such isolates of *Aspergillus versicolor* were toxigenic.

6. Sebastian A, Larson L. Characterization of the microbial community in indoor environments: a chemical-analytical approach. Applied and Environmental Microbiology 2003; 69(6): 3103-3109.

This paper is a follow-up on the use of ergosterol as a marker for fungal biomass. Integrated procedures using gas chromatography and ion trap mass spectrometry were able to show differences in house dust for a variety of compounds. This method could be used to characterize microbial communities in environmental samples. NOTE: the technology available for exposure assessment was published in the early 1990 as well as the early years of 2000. Researchers are still arguing about how to do exposure assessments.

7. Martinez KF, Rao CY, Burton NC. Exposure assessment and analysis for biological agents. Grana 2004; 43: 193-208.

This review from NIOSH focuses on exposure assessments from biological agents. This wide-ranging paper looks at microbes in water-damaged buildings together with microbes that could be used as biowarfare agents. The separation of sampling techniques versus analytical techniques includes discussion of ergosterol, a robust indicator of total fungal biomass, beta glucans, mycotoxins and endotoxins. They call for enhanced use of PCR and DNA microarray (such as discussed in the genomic sections of references in Exhibits I and II).

8. Smoragiewicz W, Cossette B, Boutard A, Krzystyniak K. Trichothecene mycotoxins in the dust of ventilation systems in office buildings. Int Arch Occup Environ Health 1993; 65: 113-117.

This 1993 paper analyzes trichothecene mycotoxins and dust samples from ventilation systems in office buildings. Duct samples were found to have at least four separate trichothecenes, confirmed by high performance liquid chromatography analysis. These were found with a detectable limit of 0.4 ng/ml of dust, consistent with prior isolation studies showing ease of detection of mycotoxins in buildings with water intrusion.

9. Tuomi T, Reijula K, Johnsson T, Hemminki K, Hintikka EL, Lindroos O, Kalso S, Koukila-Kahkola P, Mussalo-Rauhamaa H, Haajtela T. Mycotoxins in crude building materials from water-damaged buildings. Applied and Environmental Microbiology 2000; 66(5): 1899-1904.

This 2000 study from Finland demonstrated the ability to isolate 17 different mycotoxins from 79 bulk samples of interior walls from buildings in Finland with moisture problems and mold growth. Identification and numeration of fungal species present in both materials is possible.

10. Nikulin M, Pasanen AL, Berg S, Hintikka EL. *Stachybotrys atra* growth and toxin production in some building material and fodder under different relative humidities. Applied and Environmental Microbiology 1994; 60(9): 3421-3424.

This 1993 paper confirmed that *Stachybotrys* growing on building materials made sufficient quantities of toxin that could be detected by biological assays and chemical methods. Strong growth of *Stachybotrys* on wallpaper and gypsum board as well as in hay and straw resulted in satratoxin production; the toxin was not observed from *Stachybotrys* growing on wood panels.

11. Nielsen KF, Holm G, Uttrup LP, Nielsen PA. Mould growth on building materials under low water activities. Influence of humidity and temperature on fungal growth and secondary metabolism. International Biodeterioration and Biodegradation 2004; 54: 325-336.

This paper shows differences in fungal growth depending on humidity and on particular media. Maximum growth occurs at 80% humidity, but even at 5° C significant fungal growth still occurs at 90% relative humidity. Significant mycotoxin production was noted at room temperature, consistent with the hypothesis that when toxigenic fungi grow they will produce toxins.

12. Charpin-Kadouch C, Maurel G, Felipo R, Queralt J, Ramadour M, Henri D, Garans M, Botta A, Charpin D. Mycotoxin identification in moldy dwellings. Journal of Applied Toxicology 2006; 26: 475-479.

This important paper provides direct evidence for the presence of macrocyclic trichothecenes in water-damaged buildings. Fifteen buildings with floods and water damage contaminated with *Stachybotrys* or *Chaetomium* were compared to a control group of 9 buildings without water damage. There were significant differences in wall samples but not any differences for air samples. Significant correlations were observed between levels of wall surfaces and floor dust contamination.

13. Nieminen SM, Karki R, Auriola S, Toivola M, Laatsch H, Laatikainen R, Hyvarinen A, von Wright A. Isolation and identification of *Aspergillus fumigatus* mycotoxins on growth medium and some building materials. Applied and Environmental Microbiology 2002; 68(10): 4871-4875.

Using pure cultures of *Aspergillus humicotis*, production of gliotoxin was readily identified through use of selective growth media as well as on building materials. This paper was published in 2002 well before the ACOEM paper; one that insisted that such toxin formation did not occur.

14. Brasel TL, Martin JM, Carriker CG, Wilson SC, Straus DC. Detection of airborne *Stachybotrys chartarum* macrocyclic trichothecene mycotoxins in the indoor environment. Applied and Environmental Microbiology 2005-71(11): 7376-7388.

In this 2005 paper Brasel, working with Straus's group at Texas Tech, was able to readily identify airborne macrocyclic trichothecene mycotoxins in indoor environments with water damage; these toxins were not found in buildings without water damage.

15. Panaccione DG, Coyle CM. Abundant respirable ergot alkaloids from the common airborne fungus *Aspergillus fumigatus*. Applied and Environmental Microbiology 2005; 71(6): 3106-3111.

Investigators have questioned whether *Aspergillus fumigatis* becomes airborne and thereby respirable. In this paper, authors were able to show production of four separate toxins associated with conidia (smaller fragments of growing fungi). These conidia contained fungal toxins. The specific types of physical properties of conidia likely to affect their respirability were lower from *Aspergillus fumigatus* then for other toxin-formers including *Stachybotrys.*

16. Sorenson WG, Frazer DG, Jarvis BB, Simpson J, Robinson VA. Trichothecene mycotoxins in aerosolized conidia of Stachybotrys atra. Applied and Environmental Microbiology 1987; 53(6): 1370-1375.

This 1988 NIOSH paper confirms that particles made by Stachybotrys are essentially all respirable and that 85% of the dust particles were conidia and 6% were hyphal fragments. Methanol extracts from these fragments showed significant toxicity equal to that from satratoxin H and G. This paper establishes that conidia from Stachybotrys contain trichothecene mycotoxins and that inhalation of aerosols with these conidia is a potential health hazard.

17. Nielsen KF, Gravesen S, Nielsen PA, Andersen B, Thrane U, Frisvad JC. Production of mycotoxins on artificially and naturally-infested building materials. Mycopathologia 1999; 145(1): 43-56.

This 1999 paper clearly shows quantities of secondary metabolites, including but not limited to mycotoxins, made by a variety of toxin-formers. This finding is consistent with evidence that mycotoxins are not the only secondary metabolit,e made by actively-growing fungi; contributory aspects of other inflammagens must be analyzed as well.

18. Fog Nielsen K. Mycotoxin production by indoor molds. Fungal Genet Biol 2003; 39(2): 103-117.

This 2003 paper from Denmark analyzes the "worst case scenario" for homeowners - consecutive episodes of water damage provoke fungal growth and mycotoxin synthesis, with facilitated release of spores and hyphal fragments as the area dries out.

19. Brasel TL, Douglas DR, Wilson SC, Straus DC. Detection of airborne *Stachybotrys chartarum* macrocyclic trichothecene mycotoxins on particulates smaller than conidia. Applied and Environmental Microbiology 2005; 71(1): 114-122.

This 2005 paper adds to the importance of assessment of particulates found in indoor air. This paper confirms that trichothecene mycotoxins do become air borne in association with intact conidia and smaller particles independent of those toxins found on spores. Implications here are that any air sample analyzing for *Stachybotrys* or *Stachybotrys* toxins that does not include assessment of fragments down to 0.6 microns is inadequate and inaccurate.

20. Gorny RL, Reponen T, Willeke K, Schmechel D, Robine E, Boissier M, Grinshpun SA. Fungal fragments as indoor air biocontaminants. Applied and Environmental Microbiology 2002; 3522-3531.

This is one of a series of papers from Cincinnati and NIOSH; this paper looks at fungal fragments found in indoor air as being present at up to 320 times the number of spores for a given volume of air. A previous paper, (Cho 2005) found a fragment to spore ratio of 514; this paper cites 320. The actual number is of lesser importance than the fact that small particle size fungal fragments make contributions to adverse human health effects.

21. Gorny RL. Filamentous microorganisms and their fragments in indoor air-A review. Ann Agric Environ Med 2004; 11: 185-197.

Gorny follows-up his 2002 paper with this one published in 2004 showing that fragments of fungi found in indoor air are of a much greater importance than spores. This finding strongly argues against any possible consideration of dose response relationships. Gorny states a clear description of dose-response relationship with a majority of biological agents cannot be established.

22. Gorny RL, Mainelis G, Grinshpun SA, Willeke K, Dutkiewicz J, Reponen T. Release of *Streptomyces albus* propagules from contaminated surfaces. Environmental Research 2003; 91: 45-53.

This paper confirms the release of elements from *Streptomyces* from surfaces of indoor environments contaminated with the growth of these organisms. These fragments can be found in the air of the contaminated building as well as from surfaces. The indoor surfaces with resident actinomycetes therefore provide a reservoir for toxigenic elements.

23. Spaan S, Heederik DJJ, Thorne PS, Wouters IM. Optimization of airborne endotoxin exposure assessment: effects of filter type, transport conditions, extraction solutions, and

storage of samples and extracts. Applied and Environmental Microbiology 2007; 73(19): 6134-6143.

This paper attempts to provide standardization of isolation and laboratory analysis of endotoxins from indoor air environments. It confirms that endotoxins have an extremely high pro-inflammatory potency and that airborne exposure to endotoxin has been associated with respiratory tract symptoms, abnormalities in pulmonary function and systemic inflammatory effects.

24. Giovannangelo ME, Gehring U, Nordling E, Oldenwening M, van Rijswijk K, de Wind S, Hoek G, Heinrich J, Bellander T, Brunekreef B. Levels and determinants of (1-3)-β-glucans and fungal extracellular polysaccharides in house dust of (pre-) school children in three European countries. Environ Int 2007; 33(1): 9-16.

This 2007 paper confirms presence of beta-glucans and polysaccharides from fungi found in house dust in three separate European countries. This paper answers questions as to whether or not there are persistent sources of inflammation from fungi in water-damaged buildings that can be found independent of spore identification.

25. Gehring U, Douwes J, Doekes G, Koch A, Bischof W, Fahlbusch B, Richter K, Wichmann HE, Heinrich J, INGA Study Group. Indoor factors and genetics in asthma. (1-3)-β-glucan in house dust of German homes: housing characteristics, occupant behavior, and relations with endotoxins, allergens, and molds. Environ Health Perspect 2001; 109(2): 139-144.

This paper from Dr. Douwe's lab extends understanding of beta-glucans often finding those compounds in house dust in German homes. While several possible sources of exposure can raise beta-glucans, presence of mold within the home remains one of the highest potential sources correlated with increased beta-glucans.

26. Chew GL, Douwes J, Doekes G, Higgins KM, van Strien R, Spithoven J, Brunekreef B. Fungal extracellular polysaccharides, (1-3)-β-glucans and culturable fungi in repeated sampling of house dust. Indoor Air 2001; 11(3): 171-178.

This paper is a result of collaboration between US authorities Dr. Chew and Dr. Douwes, showing that beta-glucans found in house dust are good markers for presence of fungal concentrations and floor dust which can be used as a surrogate for estimating airborne fungal infections.

27. Rand TG, Giles S, Flemming J, Miller JD, Puniani E. Inflammatory and cytotoxic responses in mouse lungs exposed to purified toxins from building isolated *Penicillium brevicompactum dierckx* and *P. chrysogenum* thom. Toxicological Sciences 2005; 87(1): 213-222.

This paper looks at toxins from *Penicillium* species. The results show that the toxins commonly found on damp materials in water-damaged buildings provoke inflammatory responses.

28. Halstensen A. Species-specific fungal DNA in airborne dust as surrogate for occupational mycotoxin exposure. *Int J Mol Sci*. 2008; 9(12): 2543-58.

Methods for mycotoxin detection are not sensitive enough for the small dust masses obtained by personal sampling. Mycotoxin concentrations are highly variable, so results of PCR should be interpreted with caution.

29. Halstensen A, Nordby K, Eduard W, Llemsdal S. Real-time PCR detection of toxigenic *Fusarium* in airborne and settled grain dust and association with trichothecene mycotoxins. *J Environ Monit*. 2006; 8: 1235-1241.

Inhalational of immunomodulating mycotoxins produced by *Fusarium* may imply health risks for farmers. Mycotoxins were detected on personal samples when Fusarium was present as detected by PCR.

30. Halstensen A, Nordby, Klemsdal S, elen O, Clasen P, Eduard W. Toxigenic *Fusarium* spp. as determinants of trichothecene mycotoxins in settled grain dust. *J Occup Environ Hyg*. 2006; 3: 651-659.

PCR-detected *Fusarium* genes for trichothecenes (tri5) highly correlated with trichothecene presence.

31. Park J, Schleiff P, Attfield M, Cox-Ganser J, Kreiss K. Building-related respiratory symptoms can be predicted with semi-quantitative indices of exposure to dampness and mold. *Indoor Air*. 2004; 14: 425-433.

Semi-quantitative measures of dampness/mold exposure indices, based solely on visual and olfactory observations can predict existence of excessive building-related respiratory symptoms and diseases.

32. Rao C, Cox-Ganser J, Chew G, Doekes G, White S. Use of surrogate markers of biological agents in air and settled dust samples to evaluate a water-damaged hospital. Indoor Air 2005; 15 supple 9: 89-97.

Air and floor dust measurements of marker compounds may be better indicators of current health risk in a water-damaged environment than measurements of culturable fungi or bacteria.

A recently completed project in Sweden confirms that when testing for mycotoxins is done in WDB, those compounds are invariably found. Dr. Bloom states, "Previously it was claimed that the occurrence of mold does not necessarily mean that there are toxins present. But they are!" (**www.sciencedaily.com/releases/2008/12/081209085622.htm**, accessed 12/10/08; Bloom; Doctoral Thesis for the Department of Laboratory medicine, *Division of Medical Microbiology*, Lund University, Sweden; Mycotoxins in Indoor Environments, Determination using Mass Spectroscopy).

Microbial Growth and Fine Particles:

The papers cited below confirm the observations of Gorny and Brasel regarding fine particles released from microbial (molds and bacteria) growth in WDB. The vast majority of these particles, as measured by (1-3)-β-glucans derived form old cell walls, are less than one micron and are easily deposited in the alveolar spaces of adults and more readily, children. The older colonies shed more particulates than younger ones.

33. Cho SH, Seo SC, Schmechel D, Grinshpun SA, Reponen A. Aerodynamics and respiratory deposition of fungal fragments. Atmos Environ 2005; 39:5454-65.

Reponen T, Seo SVC, Grimsley F, Lee T, Crawford C, Grinshpun SA. Fungal fragments in mold houses: A field study in homes in New Orleans and Southern Ohio. 2007; Atmos Environ 41:8140-9.

34. Seo SC, Reponen T, Levin L, Borchelt T, Grinshpun SA. Aerosolization of particulate (1-3)-β-glucan from moldy materials. Appl Environ Microbiol 200; 74:585-93.

Seo SC, Reponen T, Levin L, Grinshpun SA. Size fractionation (1-3)-β-D-glucan concentrations aerosolized from different moldy building materials. Sci Total Environment. 2009; 407:806-14.

Animal Health: Mechanisms and Physiology

One of the tenets of the GAO report is that there exists an epidemiologic similarity between observed findings in patients and those seen in experimental subjects, animals and humans both. A reasonable question then becomes what do we see in experimental animals exposed to elements found in WDB? Fortunately, the literature on adverse health effects seen in animals is robust as the following citations demonstrate.

1. Islam Z, Harkema JR, Pestka JJ. Satratoxin G from the black mold *Stachybotrys chartarum* evokes olfactory sensory loss and inflammation in the murine nose and brain. Environ Health Perspec 2006; 224:1099-1107.

This paper clearly demonstrates that Satratoxin G instilled into the olfactory area of mice causes brain damage. The mycotoxin passes up the olfactory tract and enters the olfactory lobe causing significant damage. mRNA for the inflammatory cytokines, TNF-α, IL6, IL1, and the inflammatory protein (MIP-2) were elevated in the olfactory epithelium. In addition, apoptosis of the olfactory sensory neurons of the olfactory epithelium occurred. Marked atrophy of the olfactory nerve and glomerular layer of the olfactory bulb were reported, along with evidence of neutrophilic encephalitis.

2. Islam Z, Amuzie CJ, Harkema JR, Pestka JJ. Neurotoxicity and inflammation in the nasal airways of mice exposed to the macrocyclic trichothecene roridin A: Kinetics and potentiation by bacterial lipopolysaccharides. Toxicol Sci 2007; 98:526-41.

This is an important study regarding synergistic action of indoor contaminants. In this study, roridin A (RA) and lipopolysaccharides (LPS) were instilled into the nasal cavity of mice. As in the above referenced study, RA caused nasal inflammation damage to the olfactory epithelium and the olfactory lobe via degeneration and apoptosis. mRNA levels for pro-inflammatory cytokines (TNF-α, IL6, IL1 and MIP-2 protein were elevated. LPS augmented the adverse effects of RA.

3. Flemming J, Hudson B, Rand TG. Comparison of inflammatory and cytotoxic lung responses in mice after intrathecal exposure to two different *Stachybotrys chartarum* strains. Toxicol Sci, 2004; 78:267-75.

In this study, spores from two strains of *Stachybotrys* (atranones versus trichothecenes) were studied regarding pulmonary inflammation and cytotoxicity in mouse lungs. The authors point out that mice are more susceptible than Charles River Dawley rats to the adverse effects of the mold spores. In short, there was an increase of leakage of albumin and protein into bronchoalveolar lavage fluid compared to controls exposed to spore of *Cladosporium*. Cytotoxicity was found with increased production of TNF-α, IL-1β and IL-6. The lowest observable effect level in mice was 30 spores/gram body weight versus 3,000 spores/GW in the rats. This paper shows differential inflammatory effects following exposure of mice to trichothecenes. It also shows that when low doses are involved, spore sequestration is an important defense mechanism. This paper also shows that the no-adverse effect limit for exposure of mice to *Stachybotrys* would be the equivalent of 30 spores per gram of body for a 25 gram weight animal. This equates to

30,000 spores that would be important for a 25 kg weight animal-roughly a 60 pound child. Similarly, 150,000 spores would be the threshold for illness-effect exposure for a 125 kg animal (260 pounds). These numbers are very different from those espoused by ACOEM.

Note: The EPA, CDC and other agencies recommend the use of the "most sensitive" animal model when considering dose response and risk management. Therefore, they would suggest use of mice compared to rats in these studies.

4. Pieckova E, Hurbankova M, Cerna S, Pivovarova Z, Kovacikova Z. Pulmonary cytotoxicity of secondary metabolites of *Stachybotrys chartarum* (EHRENB.) Hughes. Ann Agric Environ Med 2006; 13:259-62.

In this study the pulmonary toxicity of the atranone-producing strain of *Stachybotrys* chartarum was investigated. The results demonstrate that atranones also cause pulmonary inflammation in Wistar rats. Intrathecally administered atranones caused a decrease in alveolar macrophage activity and an increase in the activity of the lysosomal activity of cathepsin D.

5. Rand TG, Mahoney M, White K, Oulton M. Microanatomical changes in alveolar type II cells in juvenile mice. Toxicol Sci 2002; 65:239-45.

Best summarized by the entire abstract:
Stachybotrys chartarum **is** an important environmental fungus. We have shown recently that alveolar type II cells are sensitive to exposure to *Stachybotrys chartarum* spores and to the trichothecene, isosatratoxin-F, both *in vitro* and *in vivo*, in a juvenile mouse model. This sensitivity is manifest as significant changes in the composition and normal metabolic processing of pulmonary surfactant. This study evaluated the effects of a single intratracheal exposure of *S. chartarum* spores and toxin on ultrastructure and dimensions of alveolar type II cells from juvenile mice. This was to determine whether there are concurrent morphological and dimensional changes in the alveolar type II cell that reflect the metabolic alterations in pulmonary surfactant that we observed in the treated mice. Marked ultrastructural changes were associated with alveolar type II cells in both *S. chartarum* and isosatratoxin-F treated animals compared to untreated, saline, and *Cladosporium cladosporioides* spore treated animals. These ultrastructural changes included condensed mitochondria with separated cristae, scattered chromatin and poorly defined nucleolus, cytoplasmic rarefaction, and distended lamellar bodies with irregularly arranged lamellae. Point count stereological analysis revealed a significant increase ($p < 0.05$) in lamellar body volume density in *S. chartarum* and isosatratoxin-treated animals after 48 h exposure. Mitochondria volume density was significantly lower in the isosatratoxin-F (48 h exposure) and *S. chartarum* treated (24 and 48 h exposure) animals compared to those in the other treatment groups. These results reveal that exposure to *S. chartarum* spores and toxin elicit cellular responses *in vivo* differently from those associated with exposure to spores of a nontoxigenic mold species. They also indicate that accumulation of newly secreted pulmonary surfactant in the alveolar space of *S. chartarum* and isosatratoxin- F treated animals might be a consequence of cellular trauma resulting in lamellar body volume density changes leading to increased release of pulmonary surfactant into the alveolar space.

6. J.D. Miller, M. Sun, A. Gilyan, J. Roy, T.G. Rand. Inflammation-associated gene transcription and expression in mouse lungs induced by low molecular weight compounds from fungi from the built environment. Chemico-Biological Interactions, 2010; 183(1):113-24.

This paper shows that low molecular weight compounds from various molds cause respiratory inflammation at doses relative to WDB conditions. In this study, mice were intratracheally instilled with a single dose comprising $\times 10^{-5}$ mole toxin/kg lung wt dose of either atranone C, brevianamide, cladosporin, mycophenolic acid, neoechinulin A & B, sterigmatocystin or TMC-120A. These toxins are from fungi common on damp building materials. All toxin-instilled lungs exhibited copious mucus production and alveolar macrophages with red stained cytoplasm on bronchiolar surfaces, especially at 12 h PE. Array analysis of 83 inflammation-associated genes extracted from lung tissue demonstrated a number of patterns, compared to controls. 82 genes assayed at 4 h PE and 75 genes at 12-hours post exposure (PE) were significantly altered ($p \leq 0.05$; ≥ 1.5-fold or ≤ -1.5-fold change) in the different treatment animal groups. Expression of transcriptionally-regulated genes was confirmed using immunohistochemistry that demonstrated MIP-2 and TNF-staining in respiratory bronchiolar epithelia, alveolar macrophages and alveolar type II cells. The transcriptional regulation in these genes of the treatment groups suggests that they may serve central roles in the immunomodulation of toxin-induced pro-inflammatory lung responses. Hierarchical cluster analysis revealed significant patterns of gene transcription linking the response of the toxins at equimolar doses in three groups: (1) brevianamide, mycophenolic acid and neoechinulin B, (2) neoechinulin A and sterigmatocystin, and (3) cladosporin, atranone C and TMC-120. The results further confirm the inflammatory nature of metabolites/toxins from such fungi can contribute to the development of non-allergenic respiratory health effects.

7. Kouadio JH, Dano SD, Moukha S, Mobio TA, Creppy EE. Effects of combinations of *Fusarium* mycotoxins on the inhibition of macromolecular synthesis, malondialdehyde levels, DNA methylation and fragmentation, and viability in Caco-2 cells. Toxicon 2007; 49(3): 306-317.

This work shows synergistic effects of mycotoxins on inhibition of macromolecular synthesis, DNA methylation as well as cell viability.

8. Rao CY, Brain JD Burge HA. Reduction of pulmonary toxicity of Stachybotrys chartarum spores by methanol extraction of mycotoxins. Applied and Environmental Microbiology 2000; 66(7): 2817-2821.

This 2000 paper by Rao and Burge demonstrates that toxic elements found in spores of Stachybotrys can be removed by treatment of the spores with methanol. When animals were treated with methanol-extracted spores they remain identical to control animals. When animals were treated with spores not extracted with methanol, serious adverse health effects were observed. This study of a single intense exposure to toxins containing *Stachybotrys* spores confirms causation of pulmonary inflammation and injury in a dose dependent manner. Unfortunately, there was no analysis of what toxins were found in the methanol supernatant.

9. Creasia DA, Thurman JD, Jones J, Nealley ML, York CG, Wannemacher RW, Bunner DL. Acute inhalation toxicity of T-2 mycotoxin in mice. Fundamental and Applied Toxicology 1987: 8: 230-235.

This 1987 paper from Fort Dietrich show effects of the acute inhalation of T-2 mycotoxins in both young adult and mature mice. This paper is the source of a comment that T-2 mycotoxin is at least 10 times more toxic than systemic administration and at least 20 times more toxic than dermal administration.

10. Peltola J, Ritieni A, Mikkola R, Grigoriev PA, Pocafalvi G, Andersson MA, Salkinoja-Salonen MS. Biological effects of *Trichoderma harzianum* peptaibols on mammalian cells. Applied and Environmental Microbiology 2004; 70(8): 4996-5004.

This paper uses the boar spermatozoa indicator with direct evidence of cellular toxicity from exposure to the methanol extract of *Trichoderma*. As shown by Rao, this methanol fraction contains substances that cause inflammatory changes in animals.

11. Yike I, Rand TG, Dearborn DG. Acute inflammatory responses to *Stachybotrys chartarum* in the lungs of infant rats: time course and possible mechanisms. Toxicol Sci 2005; 84(2): 407-417.

This paper shows prospectively the effects of secondary metabolites and protein factors on infant rats exposed intratracheally. Significant inflammatory responses were shown by looking at lung histology and determination of inflammatory markers. Air space was greatly reduced in the animals exposed to fungal spores compared to controls. Significant inflammatory compounds, including TNF and IL-1B were produced.

12. Rand TG, Flemming J, David Miller J, Womiloju TO. Comparison of inflammatory responses in mouse lungs exposed to atranones A and C from *Stachybotrys chartarum*. J Toxicol Environ Health A 2006; 69(13): 1239-1251.

This 2006 paper continues the work of Dr. Thomas Rand and his colleague Dr. Flemming at Saint Mary's University in Nova Scotia. There is evidence that a variety of toxic compounds made by *Stachybotrys* lead to a variety of immune, toxic, inflammatory and other pathological changes. Rand concludes that exposure to these toxins and spores of *Stachybotrys* in contaminated buildings could contribute to inflammatory lung disease onset in susceptible individuals.

13. Rosenblum Lichtenstein JH, Molina RM, Donaghey TC, Brain JD. Strain differences influence murine pulmonary responses to *Stachybotrys chartarum*. Am J Respir Cell Mol Biol 2006; 35(4): 415-423.

In this pape,r exposure by intratracheal installation of *Stachybotrys* spores into mice was performed. In this lab at Harvard, a more complete list of inflammatory markers was used showing evidence of inflammation caused by exposure to *Stachybotrys* spores. This paper showed a wide diversity of responses and a wide range of sensitivity to *Stachybotrys*. The authors conclude, "Analogous underlying genetic difference may contribute to wide range response to *Stachybotrys* among humans." This differential

genetic susceptibility is exactly what our group as shown together with collaborating physicians.

14. Penttinen P, Pelkonen J, Huttunen K, Hirvonen MR. Co-cultivation of *Streptomyces* with *Streptomyces californicus* and *Stachybotrys chartarum* stimulates the production of cytostatic compound(s) with immunotoxic properties. Toxicology and Applied Pharmacology 2006; 217: 342-351.

Researchers in Finland have long looked at interaction between actinomycetes and *Stachybotrys* as a combination of organisms in water damaged buildings that create enhanced adverse health effects. This paper confirms that these organisms synergistically enhance the cell-toxicity of either component when exposed alone.

15. Huttunen K, Pelkonen J, Nielsen KF, Nuutinen U, Jussila J, Hirvonen MR. Synergistic interaction in simultaneous exposure to *Streptomyces californicus* and *Stachybotrys chartarum*. Environmental Health Perspectives 2004; 112(4): 659-665.

Some of the same authors from the reference above subsequently have extended their research to show enhanced cytokine production from interaction of actinomycetes and *Stachybotrys*. These organisms are shown to increase adverse health effects in defined inflammatory mechanisms.

16. Murtoniemi T, Penttinen P, Nevalainen A, Hirvonen MR. Effects of microbial co-cultivation on inflammatory and cytotoxic potential of spores. Inhal Toxicol 2005; 17(12): 681-693.

This paper is one of a series of papers from Finland that has looked at actinomycetes and particular toxigenic fungi found in water-damaged buildings. There is a synergistic increase in cytotoxicity seen with these organisms. Moreover, the potency of immunotoxicity increases from this interaction over time. Said another way, the longer these organisms stay together in a water damaged building the greater the chance for significant adverse health effects.

17. Penttinen P, Huttunen K, Pelkonen J, Hirvonen MR. The proportions of *Streptomyces californicus* and *Stachybotrys chartarum* in simultaneous exposure affect inflammatory responses in mouse RAW264.7 macrophages. Inhal Toxicol 2005; 17(2): 79-85.

This is an additional paper from the Finland group looking at actinomycetes and *Stachybotrys* showing specific adverse health effects from cytokine production by particular kinds of macrophages, white blood cells that are important in the inflammatory response when these organisms grow together.

18. Zughaier SM, Zimmer SM, Datta A, Carlson RW, Stephens DS. Differential induction of the Toll-like receptor 4-MyD88-dependent and independent signaling pathways by endotoxins. Infection and Immunity 2006; 74(5): 3077.

This paper by Zughaier specifically looks at the effect of endotoxins on particular pattern receptors, elements of innate immunity that are important in generating an inflammatory

response. This basic mechanism of endotoxin association with inflammation is now well defined.

19. Hamon MA, Batsche E, Regnault B, Tham TN, Seveau S, Muchardt C, Cossart P. Histone modifications induced by a family of bacterial toxins. Proc Natl Acad Sci U S A 2007; 104(33): 13467-13472.

In addition to having effects on Toll receptors and causing systemic inflammation, endotoxins also directly activate or inhibit activity of a group of host genes. This control of "epigenetic" regulation was not well-defined previously. This 2007 paper shows the additional importance of endotoxin analysis in water-damaged buildings.

20. Medvedev AE, Kopydlowski KM, Vogel SN. Inhibition of lipopolysaccharide-induced signal transduction in endotoxin-tolerized mouse macrophages: dysregulation of cytokine, chemokine, and toll-like receptor 2 and 4 gene expression. J Immunol 2000; 164(11): 5564-5574.

This next paper extends our understanding of signaling mechanisms that are involved in development of immune responses to endotoxins.

21. Inoue KI, Takno H, Yanagisawa R, Hirano S, Sakurai M, Shimada A, Yoshikawa T. Effects of airway exposure to nanoparticles on lung inflammation induced by bacterial endotoxin in mice. Environmental Health Perspectives 2006; 114(9): 1325-1330.

This 2006 paper revisits the role of small particles, nanoparticles, on lung inflammation, and its association with bacterial endotoxin. The enhancement of inflammatory effects to bacteria endotoxin is far more prominent with smaller particles. Exposure to smaller particles can also alter coagulation parameters.

22. Medvedev AE, Lentschat A, Wahl LM, Golenbock DT, Vogel SN. Dysregulation of LPS-induced Toll-like receptor 4-MyD88 complex formation and IL-1 receptor-associated kinase 1 activation in endotoxin-tolerant cells. J Immunol 2002; 169(9): 5209-5216.

Exposure of individuals to bacterial products and bacterial toxins does not uniformly result in inflammatory effects. This paper shows the importance of particular genetic influences on tolerating exposures to endotoxins.

23. Dobrovolskaia MA, Medvedev AE, Thomas KE, Cuesta N, Toshchakov V, Ren T, Cody MJ, Michalek SM, Rice NR, Vogel SN. Induction of in vitro reprogramming by Toll-like receptor (TLR) 2 and TLR4 agonists in murine macrophages: effects of TLR "homo-tolerance" versus "hetero-tolerance" on NF-kappa B signaling pathway components. J Immunol 2003; 170(1): 508-519.

This 2003 paper is from the University of Maryland. The concept of tolerance induction is reviewed showing that there are specific genetic modulations of inflammatory cascades initiated by exposure to agents that activate Toll-like receptors. This group of compounds includes endotoxins.

24. Zhou HR, Harkema JR, Hotchkiss JA, Yan D, Roth RA, Pestka JJ. Lipopolysaccharide and the trichothecene vomitoxin (deoxynivalenol) synergistically induce apoptosis in murine lymphoid organs. Toxicol Sci 2000; 53(2): 253-263.

In 2002 the synergistic effect of bacterial products, LPS, and trichothecenes was well established to cause changes in lymph tissue of mice.

25. Islam Z, King LE, Fraker PJ, Pestka JJ. Differential induction of glucocorticoid-dependent apoptosis in murine lymphoid subpopulations in vivo following co-exposure to lipopolysaccharide and vomitoxin (deoxynivalenol). Toxicol Appl Pharmacol 2003; 182(2): 69-99.

This paper, also from Dr. Islam and Dr. Pestka at Michigan State, continues to explore the effects of simultaneous exposure to bacterial LPS and fungal toxins.

26. Islam Z, Pestka JJ. Role of IL-1beta in endotoxin potentiation of deoxynivalenol-induced corticosterone response and leukocyte apoptosis in mice. Toxicol Sci 2003; 74(1): 93-102.

This is an additional paper showing that the interaction of bacterial endotoxin and fungal toxin causes the increased release of pro-inflammatory cytokines; in this case the cytokine Interleukin 1-beta, a compound routinely observed to be abnormal in patients with acute exposure to water-damaged buildings.

27. Hirvonen MR, Huttunen K, Roponen M. Bacterial strains from moldy buildings are highly potent inducers of inflammatory and cytotoxic effects. Indoor Air 2005; 15 Suppl 9: 65-70.

This paper from Finland confirms earlier work from Dr. Pestka's lab in Michigan State in which he showed that bacterial strains from moldy buildings are potent inducers not only of inflammation but also increased cell death.

28. Martin LJ, Doebler JA, Anthony A. Scanning cytophotometric analysis of brain neuronal nuclear chromatin changes in acute T-2 toxin-treated rats. Toxicol Appl Pharmacol 1986; 85(2): 207-214.

This 1986 study in rats shows changes in brain physiology in rats exposed to T-2 trichothecene toxin treated rats

29. Martin LJ, Morse JD, Anthony A. Quantitative cytophotometric analysis of brain neuronal RNA and protein changes in acute T-2 mycotoxin poisoned rats. Toxicon 1986; 24(9): 933-941.

This 1986 paper follows-up with Dr. Martin's earlier observations of brain injury following exposure to T-2 toxin showing that the injury is not direct cell damage but involves changes in protein content.

30. Wang J, Fitzpatrick DW, Wilson JR. Effect of T-2 toxin on blood-brain barrier permeability monoamine oxidase activity and protein synthesis in rats. Food Chem Toxicol 1998; 36(11): 955-961.

T-2 toxin can cause abnormalities in blood brain-barrier in animals following injection into the abdominal cavity. This paper endorses the use of a neuropsychological evaluation instrument called RBANS that has potential benefit in assessment of metabolic changes in patients.

31. Sava V, Velasquez A, Reunova O, Sanchez-Ramos J. Acute ochratoxin-neurotoxicity: kinetics of distribution of the toxin, indices of oxidative stress and DNA repair activities in mouse brain. Accepted Abstract for Platform Presentation Society for Neuroscience, Annual Meeting Washington DC, November 2005.

This presentation on ochratoxin A shows that there are neuropathic effects of ochratoxin that can be determined within brains following exposure.

32. Bergmann F, Yagen B, Jarvis BB. The toxicity of macrocyclic trichothecenes administered directly into the rat brain. Toxicon 1992; 30(10): 1291-1294.

This paper from the early 1990's shows that trichothecenes have the capability of causing direct neuropathic effects in rats following subcutaneous injection and injection directly into the brains of these rats.

33. Young SH, Roberts JR, Antonini JM. Pulmonary exposure to 1, 3-beta-glucan alters adaptive immune responses in rats. Inhal Toxicol 2006; 18(11): 865-874.

This 2006 NIOSH paper demonstrates the increased association of exposure to beta-glucans and fungi with increased inflammatory markers found in lung. This paper is consistent with all studies done previously looking prospectively at exposure to inflammagens in that inflammatory responses can be demonstrated readily, not just in lung but systemically.

34. Robinson MJ, Sancho D, Slack EC, LeibundGut-Landmann S, Reis e Sousa C. Myeloid C-type lectins in innate immunity. Nature Immunology 2006; 7(12): 1258-1264.

This paper is an overview of the role of C-type leptins in innate immunity in which beta-glucans serve as hallmark activating compounds. These carbohydrate-detecting receptors respond rapidly to particular exposures, especially to fungi.

35. Brown GD. Dectin-1: a signaling non-TLR pattern-recognition receptor. Nature Reviews Immunology 2006; 6: 33-43.

This landmark paper on dectin-1 demonstrates the pluripotent response of the pattern recognition molecule dectin-1 that is highly involved with fungal recognition. Brown reviews the basic science that links sugar-containing compounds found on cells to activation of mannose-binding lectin pathways, which in turns lead to production of activated fragments from complement component 4 (C4a).

36. Taylor PR, Tsoni SV, Willment JA, Dennehy KM, Rosas M, Findon H, Haynes K, Steele C, Botto M, Gordon S, Brown GD. Dectin-1 is required for beta-glucan recognition and control of fungal infection. Nat Immunol 2007; 8(1): 31-38.

This recent study shows beta-glucan recognition by dectin-1 receptors and anti-fungal immunity. This study demonstrates that the non-Toll-like pattern recognition receptor is required for induction of protective immune responses. In the absence of normal control of inflammatory responses these "protected" responses can be harmful.

37. Endo Y, Takahashi M, Fujita T. Lectin complement system and pattern recognition. Immunobiology 2006; 211(4): 283-293.

The lectin/complement system is part of the primordial pattern-recognition system found in all organisms. Particular sugars are recognized by molecules that have been conserved through evolution; they are part of the first line of defense mechanism.

38. Zhu Y, Ng PM, Wang L, Ho B, Ding JL. Diversity in lectins enables immune recognition and differentiation of wide spectrum of pathogens. Int Immunol 2006; 18(12): 1671-1680.

The diversity that lectins have in their ability to recognize carbohydrates is a feature thought to compensate for the lack of acquired immunity in "primitive" organisms. Lectin binding pathways are uniquely positioned to assist in recognition of foreign antigens.

39. Huttunen K, Hyvarinen A, Nevalainen A, Komulainen H, Hirvonen MR. Production of proinflammatory mediators by indoor air bacteria and fungal spores in mouse and human cell lines. Environmental Health Perspectives 2003; 111:85-92.

This 2003 paper in Environmental Health Perspectives looks at inflammatory responses to molds and bacteria found in water-damaged buildings. By demonstrating that bacteria, fungi and actinomycetes clearly stimulate inflammatory responses, particularly from interleukins, the authors suggest that multiple participants contribute to the development of illness in patients exposed to water-damaged buildings.

40. Ochiai E, Kamei K, Hiroshima K, Watanabe A, Hashimoto Y, Sato A, Ando A. The pathogenicity of *Stachybotrys chartarum*. Jpn J Med Mycol 2005; 46: 109-117.

This 2005 paper is an overview of *Stachybotrys*; it should be compared to the paper produced by Ghannoum discussed in the "Nay-Sayers" section of this statement. The conclusions here are clear: (i) inhalation of conidia caused significant inflammatory responses in the lung following inhalation and that (ii) inflammation causes serious damage.

41. Netea MG, Van der Meer JWM, Sutmuller RP, Adema GJ, Kullberg BJ. From the Th1/Th2 paradigm towards a Toll-like receptor/T-helper bias. Antimicrobial Agents and Chemotherapy 2005; 49(10): 3991-3996.

This paper from Netea is a classic overview of immune responses, including Toll-like receptors, to inflammatory agents found in fungal elements and fungi.

42. Rand TG, Flemming J, Miller JD, Womiloju TO. Comparison of inflammatory responses in mouse lungs exposed to atranones A and C from *Stachybotrys chartarum*. Journal of Toxicology and Environmental Health 2006; 69: 1-13.

This paper is from the Canadian researcher, Dr. Rand. He has looked at dose-response relationships to a variety of fungal trichothecenes instilled into mice. There are different kinds of fungal toxins in the trichothecene family. Although this work shows that even though there are differences in results of inflammation from toxins made by *Stachybotrys*, they all cause inflammation that can contribute to disease in susceptible individuals.

43. Rand TG, Giles S, Flemming J, Miller JD, Puniani E. Inflammatory and cytotoxic responses in mouse lungs exposed to purified toxins from building isolated *Penicillium brevicompactum dierckx* and *P. chrysogenum thom*. Toxicological Sciences 2005; 87(1): 213-222.

This paper demonstrates that toxins produced by *Penicillium* species are commonly found on damp materials in water-damage buildings and that they provoke toxin inflammatory responses.

44. Donohue M, Wei W, Wu J, Zawia NH, Hud N, De Jesus V, Schmechel D, Hettick JM, Beezhold DH, Vesper S. Characterization of nigerlysin C, hemolysin produced by *Aspergillus niger*, and effect on mouse neuronal cells in vitro. Toxicology 2006; 219: 150-155.

This paper from EPA shows that a hemolysin, called nigerlysin, made by *Aspergillus niger*, stimulates inflammatory responses that cause damage to mouse neurons. This is an additional mechanism by which adverse neurologic and neurocognitive effects occur following exposure to water-damaged buildings.

45. Rao CY, Burge HA, Brain JD. The time course of responses to intratracheally instilled toxic *Stachybotrys chartarum* spores in rats. Mycopathologia 2000; 149: 27-34.

This is the infamous paper mis-used by ACOEM. Washed spores with unknown content of toxin were instilled into rat trachea hyperacutely. Significant inflammatory responses were observed beginning within six hours following exposure. This paper specifically says that the animals were exposed to relatively large numbers of spores at one time but that "realistic exposures are probably chronic and at low concentrations." In a study of multiple and intranasal exposures over three weeks in mice there was evidence of severe inflammatory changes in hemorrhage and bronchioles. Rao also noted evidence of hemorrhages. Rao concludes that direct pulmonary exposure to *Stachybotrys* spores can cause severe inflammatory effects in lungs. The author calls for investigation in chronic exposure situations to assess the risk to humans. Note this paper did not review any of the known inflammatory markers involving inflammatory cytokines or complement, instead analyzing markers with no specificity.

46. Romani L. Immunity to fungal infections. Nature Reviews Immunology 2004; 4: 11-23.

This is a 2004 review of inflammatory responses to infecting fungi. It discusses innate immunity, Toll receptors, inflammatory cytokines, complement, dendritic cells and differential gene activity as elements of innate immune response. This paper stands as a testament to the pluripotent inflammatory responses of exposure to fungi.

47. Vesper SJ, Vesper MJ. Possible role of fungal hemolysins in sick building syndrome. Advances in Applied Microbiology 2004; 55: 191-213.

The paper is from the EPA. Dr. Vesper has been looking at fungal hemolysins found indoors in water-damaged buildings for a number of years. This paper is a careful review of hemolysins as a source of inflammatory response.

48. Jussila JJ. Inflammatory responses in mice after intratracheal instillation of microbes isolated from moldy buildings. PhD dissertation 01/24/03 National Public Health Institute, Finland.

This PhD dissertation from 2003 provides an extended overview of inflammatory responses found in mice after intratracheal installation of microbes, actinomycetes, bacteria and fungi isolated from moldy buildings.

49. Mason CD, Rand TG, Oulton M, MacDonald JM, Scott JE. Effects of *Stachybotrys chartarum* (atra) conidia and isolated toxin on lung surfactant production and homeostasis. Natural Toxins 1998; 6: 27-33.

This ten-year-old paper looked at the effects of *Stachybotrys* conidia and trichothecene toxins on metabolic functioning of cells that line alveolar spaces, called alveolar type-2 cells. Significant depression of cell function was noted.

50. Nikulin M, Reijula K, Jarvis BB, Veijalainen P, Hintikka EL. Effects of intranasal exposure to spores of *Stachybotrys atra* in mice. Fundamental and Applied Toxicology 1997; 35: 182-188.

This paper has been misquoted frequently in litigation by defense consultants. Nikulin shows that low levels of spores instilled in mice produce significant inflammatory responses when evaluated over three weeks. These inflammatory responses were less prominent in mice exposed to spores from non-toxin formers but they still had inflammatory responses.

51. Wilkins CK, Larsen ST, Hammer M, Poulsen OM, Wolkoff P, Nielsen GD. Respiratory effects in mice exposed to airborne emissions from *Stachybotrys chartarum* and implications for risk assessment. Pharmacology & Toxicology 1998; 83: 112-119.

This ten-year-old paper looks at health effects in mice exposed to *Stachybotrys*. The study quotes that many factors are important for the transport of biologically active mold metabolites from building materials to occupants. No direct relationship between immediate biological effects and surface area covered with mold can be concluded. “Risk assessments should be based on estimated effects of emitted vapors, effects of liberated

particles, sensitization potentials of mold spores and effects of generated metabolites (mycotoxins)."
52. Shimizu T, Kida Y, Kuwano K. *Mycoplasma pneumoniae*-derived lipopeptides induce acute inflammatory responses in the lungs of mice. Infection and Immunity 2008; 76(1): 270-277.

Inflammatory responses in lungs of animals are defined following exposure to antigens from microbes.

53. Rand TG, Miller JD. Immunohistochemical and immunocytochemical detection of SchS34 antigen in *Stachybotrys chartarum* spores and spore-impacted mouse lungs. Mycopathologia 2008; 165: 73-80.

Toxins can cause increased production of particular enzymes by pathogen-associated molecular pattern receptors found in cells that line the lungs. These cellular functions provide a differential capacity to respond to cell wall components and play a crucial role in innate immune response airways.

54. Wang T, Moreno-Vinasco L, Huang Y, Lang G, Linares J, Goonewardena S, Grabavoy A, Samet J, Geyh A, Breyssse P, Lussier Y, Natarajan V, Garcia J. Murine lung responses to ambient particulate matter: genomic analysis and influence of airway hyper-responsiveness. Environmental Health Perspectives 2008; 116: 1500-1508.

Particulate matter evokes pro-inflammatory responses that contribute to asthma susceptibility.

55. Chang ZL, Netski D, Thorkildson P, Kozel TR. Binding and internalization of glucuronoxylomannan (GXM), the major capsular polysaccharide of *Cryptococcus neoformans*, by murine peritoneal macrophages. Infect Immun 2006; 74: 144-151.

Factors important in host responses GXM include cell wall components that are recognized and internalized by white blood cells, an essential step leading to activation of additional inflammatory cascades.

56. Bloom E. PhD dissertation. Mycotoxins in indoor environments. 2008. Department of Laboratory Medicine. Division of Medical Microbiology Land University, Sweden.

Most complete discussion of invariant finding of mycotoxins in WDB if fungi are found.

57. Penttinen P, Huttunen K, Pelkonen J, Hirvonen M. The proportions of *Streptomyces californicus* and *Stachybotrys chartarum* in simultaneous exposure affect inflammatory responses in mouse RAW264.7 macrophages. Inhal Toxicol 2005; 2: 79-85.

Adverse health effects from WDB arise from an exposure consisting of a complex interaction between microbes and indoor pollutants. Co-exposure to fungi and actinomycetes enhance the adverse effects of one or the other taken singly.

58. Thorgersen E, Pharo A, Haverson K, Axelsen A, Gaustad P, Kotwal G, Mollnes S.

Inhibition of complement and CD14 attenuates the *Escherichia coli*-induced inflammatory response in porcine whole blood. Infect Immun 2009; 2: 725-32. Blockade of complement reduces inflammation caused by ***E. coli*** but also reduced bacterial clearance.

59. Huang H, Ostroff G, Lee C, Wang J, Specht C, Levitz S. Distinct patterns of dendritic cell cytokine release stimulated by fungal B-glucans and toll-like receptor agonists. Infection and Immunity 2009; Vol. 77: 1744-1781.

B-glucans have distinct effects on cytokine responses following dendritic cell stimulation by different Toll agonists. These patterns of responses skew immune responses to fungi.

60. Yike I, Rand T, Dearborn D. Acute inflammatory responses to *Stachybotrys chartarum* in the lungs of infant rats: time course and possible mechanisms. Toxicol Sci 2005; 2: 408-17.

Fungal proteins are involved in the inflammatory response to *Stachybotrys* and shed new light on the clinical importance of indoor fungi.

61. Ramaprakash H, Ito T, Standiford T, Kunkel S, Hogaboam C. Toll-like receptor 9 modulates immune responses to *Aspergillus fumigatus* conidia in immunodeficient and allergic mice. Infection and Immunity 2009; 108-119.

Taken together, these data suggest that TLR9 modulates pulmonary antifungal immune responses to conidia through dectin-1 expression.

62. Miller JD, Sund M, Gilyan A, Roy J, Rand TG. Inflammation-associated gene transcription and expression in mouse lungs induced by low molecular weight compound from fungi from the built environment. Chemico-Biol Interactions 2009;

Mice were given intrathecal mold spores from different genera of indoor molds at concentrations usually observed growing on building materials. The results show that transcriptional genes may be responsible for the pro-inflammatory response to toxins. The toxins tested were atranone C, brevianmide, cladosporin, mycophenolic acid, neoechinulin A & B, sterigmatocystin and TMC 120 A. The results demonstrate that health concerns are present with respect to fungi other than *Stachybotrys chartarum.*

63. Pieckova E, Hurbankova M, Cerna S, Liskova A, Cerna S, Liskova A, Kaovacikova Z, Kollikakova Z, Wimmerova A. Inflammation and haematotoxic potential of indoor *Stachybotrys chartarum* (EHRENB.) Hughes metabolites. Arh Hig Rada Toksikol 2009; 60:401-09.

Rats were treated intrathecally with DMSO (control), endo-metabolites (metabolites present in fine particles), exo-metabolites (spore mycotoxins) and DAS (control) and then examined by BALF and by autopsy at sacrifice. Both endo- and exo-metabolites caused an increase in inflammatory cells in BALF along with a decrease in alveolar macrophages. The rats also had a significant drop in RBCS, RBC hemoglobin concentration and hematocrit. Thus, endo-metabolites of fine particles are also toxic and adversely affect RBCs. The authors conclude "**Due to hematotoxic and inflammation-**

inducing properties, metabolites of *S. chartarum* can cause damage to the airways and haematological disorders in occupants of mouldy dwellings (emphasis added).” This is an important paper in that it shows that the metabolites present in fine particles have potential adverse effects on either erythrogenesis and/or hemolysis.

64. Roda E, Coccini T Acerbi D, Castoldi AF, Manzo L. Comparative in vitro and ex-vivo myelotoxicity of aflatoxins B1 and M1 on hematopoietic progenitors (BFU-E, CFU-E, and CFU-GM): species-related susceptibility. Toxicology in Vitro 2020; 24:217-23.

The authors investigated the adverse affects of aflatoxins on human and mouse bone marrow progenitor cells cultured in vivo. The control mice were injected with aflatoxin and studied for acute effects. The observations clearly show the following: (1) Human bone marrow (BM) progenitor cells were more sensitive (about 4 times) than mouse BM progenitors to both toxins, particularly CFU E erythroid progenitors) and CFU-GM (myeloid precursors); 2) Irrespective of animal species, both AFB1 and AFM1 affected more markedly both the myeloid lineage (CFU-GM) and the immature erythroid progenitors (BFU-E) than the more mature DVU-E colonies; and 3) Acute, in vivo toxicity of toxicity was not observed on either cell line. Ex-vivo studies are now accepted as means to study the adverse affects of chemicals on bone marrow differentiation. The data clearly show that human BM progenitors are more sensitive to the toxic affects of aflatoxins than are mouse BM. The adverse affects were observed in vivo at the ppt to low ppb ranges. Of note, assessment of acute changes bears a reduced relevance when compared to those changes seen chronically in patients with CIRS-WDB.

Human Health Effects

Actual research studies done on humans have shown a consistent pattern of illness symptoms identified following exposure to the interior environment of WDB. Over 40 studies, including over 50,000 patients from 14 countries published since the IOM report, show that the illness is recognized globally. Those who would disagree with the concept that exposure to WDB can cause illness ("Nay-Sayers") are unable to provide evidence of a *single* human research study that includes physiologic parameters showing that no illness exists following exposure to WDB. In the absence of any studies refuting the presence of illness, and the multiple studies demonstrating presence of illness, each showing epidemiologic similarity one to the other, the arguments against human illness from WDB are hollow and unscientific.

Moreover, prospective studies that have looked at patients with illness treated successfully with pharmacologic interventions who then were (1) exposed to all other known environments in their day-to-day life other than the WDB with no evidence of change in symptoms, VCS or laboratory findings; but those same patients, when (2) evaluated each day for three consecutive days without use of protective medications following exposure to a known WDB show a sequential relapse in inflammatory parameters, with C4a, a split product of complement activation, rising on day 1; leptin rising on day 2; MMP9 rising between day 2 to day 3; VEGF rising on day 1 and falling by day 3; TGF β-1 rising on day 1, though somewhat later than C4a; Factor VIII falling on day1, but recovering by day 3; von Willebrand's factor and antigen falling by day 3, with bleeding (epistaxis or hemoptysis), if it occurs, seen as ristocetin-associated cofactor falls and von Willebrand's multimers decrease. TGF β-1 rises rapidly with re-exposure and may remain elevated for weeks after such a rise. Reacquisition of illness is shown by symptoms, VCS and laboratory findings to equal in three days what may have taken months or years to develop. What these findings show is the similarity of host innate immune responses across populations; these findings do not demonstrate a monotonic dose response. They demonstrate an exponentially expanding immunologic response to contaminants found in the indoor air of WDB.

These immunologic findings confirm that the illness is an acute and chronic inflammatory response syndrome with genetic susceptibility; cytokine responses; Th-17 cellular responses; complement responses; hypoxia inducible factor responses and coagulation system responses. These finding also show that correction of the dense inflammatory illness must begin with removal from exposure and institution of a toxin-binding medication. Correction of toxin carriage is therefore necessary as an initiating step, but by itself is not always sufficient to correct the CIRS-WDB.

In addition, the epidemiologic similarity of patients with illness at baseline is shown by five studies in adults (see references from Shoemaker et al, page 160) that look at inflammatory parameters in patients. There is no significant difference between physiologic abnormalities found in cases in all studies, but these findings are wholly disparate from findings in control patients. One large study of pediatric patients at

baseline showed the same kinds of inflammatory and autoimmune parameters in adults (ref).

Taken as a whole, when we put together the findings of (1) the IOM study that declared that there were cytokine and genetic factors found in cases; (2) the GAO report that discussed the immunologic and inflammatory basis of the illness, with a genetic component; and (3) the WHO report that emphasized the inflammatory, immunologic and genetic bases for the illness; with the human health studies cited herein that show vast differences in measurements of inflammation based on known immunologic (host) responses that have genetic basis, we have clear evidence of internal consistency of the medical science that underlies adverse human health effects seen in patients made ill by exposure to the interior environment of WDB.

We also note the AIHA Green book (Chapter one) states: "The worldwide consensus of scientific opinion is that the contaminants found in WDB are scientifically proven to cause human health effects."

1. Kouadio JH, Mobio TA, Baudrimont I, Moukha S, Dano SD, Creppy EE. Comparative study of cytotoxicity and oxidative stress induced by deoxynivalenol, zearalenone or fumonisin B1 in human intestinal cell line Caco-2. Toxicology 2005; 213(1-2): 56-65.

Toxins disrupt sphingolipid metabolism as well as disrupting lysosomes. These factors are important in acetylation, discussed in section 30.

2. Kovacikova Z, Tatrai E, Pieckova E, Tulinska J, Pivovarova Z, Matausic-Pisl M, Kuricova M, Wsolova L. An in vitro study of the toxic effects of *Stachybotrys chartarum* metabolites on lung cells. ATLA 2007; 35: 47-52.

Isolates of *Stachybotrys* showed direct toxic effects on lung cells including alveolar macrophages and epithelial cells. Following exposure there were changes in the production of inflammatory markers, including TNF, and a reduction of antioxidant compounds.

3. Tsunawaki S, Yoshida LS, Nishida S, Kobayashi T, Shimoyama T. Fungal metabolite gliotoxin inhibits assembly of the human respiratory burst NADPH oxidase. Infection and Immunity 2004; 72(6): 3373-3382.

Aspergillus toxins including fumagillin (well-studied in the angiogenesis literature), and gliotoxin each selectively impair particular metabolic pathways that normally are used by the unaffected host to generate energy.

4. Saenz JB, Doggett TA, Haslam DB. Identification and characterization of small molecules that inhibit intracellular toxin transport. Infection and Immunity 2007; 75(9): 4552-4561.

Part of the question of toxin effect and impact on cellular genomics has been the question of how toxins obtain entry into cells. This 2007 paper shows that small toxins readily

reach intercellular destinations following endocytosis. This mechanism is important when considering the role of the endocytosis associated with mannose receptors discussed in section 27.

5. Meloche JL, Smith TK. Altered tissue amino acid metabolism in acute T-2 toxicosis. Proc Soc Exp Biol Med 1995; 210(3): 260-265.

T-2 toxin is a *Fusarium* trichothecene that alters brain neurochemistry. Exposure to this toxin reduced uptake of a particular amino acid, leucine.

6. Yike I, Distler Am, Ziady AG, Dearborn DG. Mycotoxin adducts on human serum albumin: biomarkers of exposure to *Stachybotrys chartarum*. Environmental Health Perspectives 2006; 114(8): 1221-1226.

This paper from Dearborn's laboratory confirms the presence of satratoxin G, one of the trichothecenes made by *Stachybotrys*, bound to albumin in blood. The "adducts" of satratoxin G and albumin were found in human and animals exposed to *Stachybotrys* but not found in humans and animals not exposed to *Stachybotrys*.

7. Brasel TL, Campbell AW, Demers RE, Ferguson BS, Fink J, Vojdani A, Wilson SC, Straus DC. Detection of trichothecene mycotoxins in sera from individuals exposed to *Stachybotrys chartarum* in indoor environments. Archives of Environmental Health 2004; 59(6): 317-323.

This paper published with Dr. Fink's group in Wisconsin confirms that trichothecene and mycotoxins can be found in blood of individuals exposed to indoor environments with water damage and growth of *Stachybotrys*. These compounds were not found in patients that did not have exposure to such buildings. The technology used, QuantiTox from EnviroLogix, was the same that Dr. Dearborn used in his *Stachybotrys* paper.

8. Wannemacher RW, Wiener SL. Trichothecene Mycotoxins. Textbook of Military Medicine: Medical Aspects of Chemical and Biological Warfare: Chapter 34.

This important chapter underscores the military significance of mycotoxins both from prior use together with concerns for use as weapons. While civilians may argue the role of mycotoxins in human health, the military already knows about it.

9. Rosenblum Lichtenstein JH, Molina RM, Donaghey TC, Brain JD. Strain differences influence murine pulmonary responses to *Stachybotrys chartarum*. Am J Respir Cell Mol Biol 2006; 35(4): 415-423. Note this paper cited in animal studies section as well.

In this paper prospective exposure in intratracheal installation of *Stachybotrys* spores into mice was again performed. At this Harvard lab, a more complete list of inflammatory markers was used showing evidence of inflammation caused by exposure to *Stachybotrys* spores. This paper showed diversity and a wide range of sensitivity to *Stachybotrys*. The authors conclude, "Analogous underlying genetic difference may contribute to wide range response to *Stachybotrys* among humans." This differential genetic susceptibility is exactly what our group as shown together with collaborating physicians.

10. Hodgson MJ, Morey P, Leung WY, Morrow L, Miller D, Jarvis BB, Robbins H, Halsey JF, Storey E. Building-associated pulmonary disease from exposure to *Stachybotrys chartarum* and *Aspergillus versicolor*. J Occup Environ Med 1998; 40(3): 241-249.

This paper written by Hodgson and Storey is another discussion of individuals made ill by exposure to a water-damaged courthouse in Florida. In the paper the authors show presence of satratoxin G and H together with cytotoxic laboratory analyses showing the differences between cases and controls.

11. Jarvis BB, Miller JD. Mycotoxins as harmful indoor air contaminants. Appl Microbiol Biotechnol 2005; 66(4): 367-372.

This overview of mycotoxins contains a discussion in terms of human health effects of exposure.

12. Casadevall A, Pirofski LA. The weapon potential of human pathogenic fungi. Medical Mycology 2006; 44: 689-696.

Published in 2006 discusses the very clear potential that indifference to fungi as a potential biological weapon against human populations is a problem engendered by lack of infectious aspect, lack of history of use for development of biowarfare weapons and a low understanding in current literature of what complications of exposure are.

13. Sidell FR, Takafuji ET, Franz DR. Medical aspects of chemical and biological warfare. 1997; NLM Unique ID: 9709389.

This paper clearly shows the extreme interest of the military in the biowarfare weapon potential of fungal toxins.

14. Bruel KF, Kougias P, Rice PJ, Wei D, De Ponti K, Wang J, Laffan JJ, Li C, Kalbfleisch J, Williams DL. Anterior pituitary cells express pattern recognition receptors for fungal glucans: implications for neuroendocrine immune involvement in response to fungal infections. Neuroimmunomodulation 2004; 11(1): 1-9.

This paper provides a mechanism for linking the inflammatory response and fungal glucans to changes of effect of pituitary hormone function.

15. Taylor MJ, Smart RA, Sharma RP. Relationship of the hypothalamic-pituitary-adrenal axis with chemically induced immunomodulation. I. Stress-like response after exposure to T-2 toxin. Toxicology 1989; 56(2): 179-195.

This 1989 paper looks at the changes of the hypothalamic pituitary axis following immunomodulation stimulated by exposure to a trichothecene mycotoxin, T-2.

16. Gonzalez-Rey E, Chorny A, Delgado M. Regulation of immune tolerance by anti-inflammatory neuropeptides. Nature Reviews Immunology 2007; 7: 52-63.

This paper summarizes the dominant role of anti-inflammatory neuropeptides including MSH in regulating cytokine responses that in turn are linked to changes in hormonal function. This paper also cites VIP as a central element together with MSH controlling regulatory T-cells.

17. Panaccione DG, Coyle CM. Abundant respirable ergot alkaloids from the common airborne fungus *Aspergillus fumigatus*. Applied and Environmental Microbiology 2005; 71(6): 3106-3111.

This 2005 paper continues the series of peer-reviewed papers that demonstrate a high concentration of toxins associated with conidia, including those from *Aspergillus*, which contribute to the toxin burden and contribute to adverse health effects associated with exposure to these compounds.

18. Douwes J, Siebers R, Wouters I, Doekes G, Fitzharris P, Crane J. Endotoxin, (1->3)-β-D-glucans and fungal extra-cellular polysaccharides in New Zealand homes: a pilot study. Ann Agric Environ Med 2006; 13(2): 361-365.

This paper continues the theme that endotoxin interaction with other elements in water-damaged buildings contributes to adverse human health effects. Presence of endotoxins and beta-glucans were found to be higher in homes with water damage.

19. Gandara A, Mota LC, Flores C, Perez HR, Green CF, Gibbs SG. Isolation of *Staphylococcus aureus* and antibiotic-resistant *Staphylococcus aureus* from residential indoor bioaerosols. Environmental Health Perspectives 2006; 114(12): 1859-1864.

Presence of unusual coagulase negative staph is important in our clinical work with patients exposed to water-damaged buildings. This paper confirms that antibiotic resistant staphylococci are found within residential homes.

20. Anyanwu EC, Kanu I, Nwachukwu NC, Saleh MA. Chronic environmental exposure to *Alternaria tenuis* may manifest symptoms of neuropsychological illnesses: A study of 12 cases. J Appl Sci Environ Mgt 2005; 9(3): 45-51.

This 2005 paper confirms that chronic exposure to toxigenic molds can lead to neuropsychological effects.

21. Schoental R. Fusarial mycotoxins and behaviour: possible implications for psychiatric disorder. Br J Psychiatry 1985; 146: 115-119.

This 1985 paper from England shows that the central nervous system is a target for mycotoxins and neurological effects follow such exposure.

22. Baldo JV, Ahmad L, Ruff R. Neuropsychological performance of patients following mold exposure. Appl Neuropsychol 2002; 9(4): 193-202.

Exposure to mold resulted in neuropsychological data showing impairment to under the 10^{th} percentile in a number of cognitive measures with the most consistent deficits being

in visuo-spatial learning, visuo-spatial memory, verbal learning and followed by motor speed.

23. Kilburn KH. Indoor mold exposure associated with neurobehavioral and pulmonary impairment: a preliminary report. Arch Environ Health 2003; 58(7): 390-398.

Also: Kilburn KH. Neurohavioral and pulmonary impairments in 105 adults with indoor exposure to molds compared to 100 exposed to chemicals. Toxicol Indust Health 2009; 25:681-92.

Dr. Kilburn has looked at mold patients for many years in his role as a full professor at the Keck School of Medicine at the University of Southern California. He is able to show a total of 21 neuropsychological functions that are abnormal in patients with exposure to water-damaged buildings not found in controls.

24. Empting LD. Neurologic and neuropsychiatric syndrome features of mold and mycotoxins exposure. Toxicol Indust Health 2009; 25:81.

Dr. Empting has evaluated several patients referred to him by Dr. Donald Dennis for neurological evaluation. Dr. Empting reports that individuals in WDB can have one or more neurological problems such as: migraine and atypical pain; pharyngitis and neuralgia; head and neck myalgias; inflammation induction of distant pain; balance and ataxic disorders of movement; and diffuse neuropsychiatric syndrome associated with exposure.

25. Dennis D, Robertson D, Curtis C, Black J. Fungal exposure endocrinopathy in sinusitis with growth hormone deficiency. Toxicol Indust Health 2009; 25:669-80.

In this paper Dr. Dennis and colleagues review their treatment protocol for WDB patients with chronic fungal rhinosinusitis. These patients also had chronic fatigue as a result of hypopituitarism and deficiency of growth hormone and thyroid insuffiency. Treatment included intranasal glutathione, antifungals and intranasal corticosteroids. This paper extends the earlier observations of Dr. Dennis published in Molds and Mycotoxins (KH Kilburn, Ed.) Heldref Publications, 2004.

26. Rea WJ, Didriksen N, Simon TR, Pan Y, Fenyves EJ, Griffiths B. Effects of toxic exposure to molds and mycotoxins in building-related illnesses. Arch Environ Health 2003; 58(7): 399-405.

Dr. Rea has looked at patients exposed to mold for many years. He shows objective abnormalities in autonomic nervous system testing in 100 patients with mold exposure.

27. Gordon WA, Cantor JB, Johanning E, Charatz H, Ashman TA, Breeze JL, Haddad L, Abramowitz S. Cognitive impairment associated with toxigenic fungal exposure: a replication and extension of previous findings. Applied Neuropsychology 2004; 11(2): 65-74.

Dr. Wayne Gordon has worked with Dr. Eckardt Johanning for a number of years on patients exposed to water-damaged buildings. Dr. Gordon has shown specific

neuropsychological data and symptom reports on 31 individuals with exposure to water-damaged buildings. Significant evidence of traumatic brain injury was found as indicated by essential correlations of patients with mold exposure compared to those with known traumatic brain injury.

28. Dangman KH, Bracker AL, Storey E. Work-related asthma in teachers in Connecticut schools with chronic water damage and fungal growth. Connecticut Med 2005; 69:9-17.

These authors reviewed a series of 55 teachers from schools in Connecticut who presented consecutively to their clinic with work-related disease. The authors concluded, "In summary, the present data suggest that working in chronically damp and/or moldy-laden school buildings is associated with development of (a) upper-respiratory symptoms, (b) new-onset asthma, and perhaps, (c) granulomatous lung disease. It is reasonable to conclude that work-place exposures in water-damaged school buildings led to deleterious effects on the health of our patients who worked in them and teachers may not necessarily be a low-risk group for developing work-related or occupational asthma."

29. Crago BR, Gray MR, Nelson LA, Davis M, Arnold L, Thrasher JD. Psychological, neuropsychological, and electro-cortical effects of mixed mold exposure. Archives of Environmental Health 2004; 452-462.

This paper suggests the possibility that dose-response relationship between measures of mold exposure and abnormal neuropsychological testing can be compared to QEEG measures that confirm the role of exposure to toxigenic molds on cognitive affects.

30. Gordon KE, Masotti RE, Waddell WR. Tremorgenic encephalopathy: a role of mycotoxins in the production of CNS disease in humans? Can J Neurol Sci 1993; 20: 237-239.

This paper is a case report from Canada in 1993 that demonstrates abnormalities of neurologic function including dementia and development of tremor following exposure to moldy silage.

31. Cooley JD, Wong WC, Jumper CA, Straus DC. Correlation between the prevalence of certain fungi and sick building syndrome. Occup Environ Med 1998; 55: 579-584.

This early paper from Texas Tech shows that bioaerosols found in 48 separate schools with evidence of water damage were associated with human health effects, including cognitive effects. Dr. Straus's group in this paper does not follow-up on specific brain injury symptoms.

32. Willment JA, Marshall AS, Reid DM, Williams DL, Wong SY, Gordon S, Brown GD. The human beta-glucan receptor is widely expressed and functionally equivalent to murine dectin-1 on primary cells. Eur J Immunol 2005; 35(5): 1539-1547.

This is one of a series of papers from Dr. Brown's laboratory in Oxford, England. In this paper dectin-1 acts as a major beta-glucan receptor on particular white blood cells

including macrophages, dendritic cells, neutrophils and eosinophils. Dectin-1 activation contributes to the inflammatory response to carbohydrates from white blood cells.

33. Valera I, Vigo AG, Alonso S, Barbolla L, Crespo MS, Fernandez N. Peptidoglycan and mannose-based molecular patterns trigger the arachidonic acid cascade in human polymorphonuclear leukocytes. J Leukoc Biol 2007; 81(4): 925-933.

This 2007 paper demonstrates that in addition to activation of complement following activation of pattern recognition receptors by particular carbohydrates, there is activation of an additional pathway, the arachidonic acid pathway. These papers taken together in this section show the diversity and accentuation of cascading inflammatory responses to a single stimulus.

34. Douwes J, van der Sluis B, Doekes G, van Leusden F, Wijnands L, van Strien R, Verhoeff A, Brunekreef B. Fungal extracellular polysaccharides in house dust as a marker for exposure to fungi: relations with culturable fungi, reported home dampness, and respiratory symptoms. J Allergy Clin Immunol 1999; 103(3 Pt 1): 494-500.

Polysaccharides, "sugars," in house dust are markers for exposure to fungi. Presence of these fungal extracellular products correlates with dampness and respiratory symptoms.

35. Jiang H, Chess L. Regulation of immune responses by T cells. N Engl J Med 2006; 354: 1166-1176.

This overview of inflammatory responses from T-cells looks at the cellular basis of inflammation; these principles are active in all aspects of inflammatory responses to elements found in water-damaged buildings. This paper also discusses the differential changes involved in autoimmunity presented by different HLA haplotypes.

36. Nurkiewicz TR, Porter DW, Barger M, Millecchia L, Rao MK, Marvar PJ, Hubbs AF, Castranova V, Boegehold MA. Systemic microvascular dysfunction and inflammation after pulmonary particulate matter exposure. Environmental Health Perspectives 2006; 114(3): 412-419.

There is lack of microvascular control of peripheral resistance following exposure to particulates. This paper looks at vascular responses in addition to inflammatory responses.

37. Marc MM, Korosec P, Kosnick M, Kern I, Flezar M, Suskovic S, Sorli J. Complement factors C3a, C4a, and C5a in chronic obstructive pulmonary disease and asthma. American J of Respir Cell and Molecular Biology 2004; 31: 31-33.

Split products of complement activation, including C3a and C4a, are highly important in evaluating changes in pulmonary physiology suggestive of inflammatory contribution in asthma and chronic lung disease.

38. Kelk P, Claesson R, Hanstrom L, Lerner UH, Kalfas S, Johansson A. Abundant secretion of bioactive IL-1β by human macrophages induced by *Actinobacillus actinomycetemcomitans* leukotoxins. Infection and Immunity 2005; 73(1): 453-458.

Paralleling the findings of inflammatory responses to toxins made by bacteria, fungi and actinomycetes, an organism was found in oral cavities producing a toxin that selectively kills white blood cells. Part of the mechanism of action is the triggering of abundant production and secretion of active IL-1β.

39. Kohl J, Baelder R, Lewkowich IP, Pandey MK, Hawlisch H, Wang L, Best J, Herman NS, Sproles AA, Zwirner J, Whirsett JA, Gerard C, Sfyroera G, Lambris JD, Wills-Karp M. A regulatory role for the C5a anaphylatoxins in type 2 immunity in asthma. Journal of Clinical Investigation 2006; 116(3): 783-796.

The complement split product, C5a, has a significant role in promoting inflammatory problems in the lung that can develop into what becomes diagnosed as asthma.

40. Desjardins AE. Trichothecenes: from yellow rain to green wheat. ASM News 2003; 69(4): 182-185.

This paper from the USDA Agricultural Research Service in Peoria looks at trichothecenes as fungal toxins capable of being used in bioterrorism and biowarfare. This paper summarizes prior use of toxins on people, causing injury.

41. Vesper SJ, Vesper MJ. Stachylysin may be a cause of hemorrhaging in humans exposed to Stachybotrys chartarum. Infection and Immunity 2002; 70(4): 2065-2069.

This earlier paper from Dr. Vesper from the EPA shows the importance of another hemolysin, called stachylysin, causing inflammatory responses and hemorrhage in patients exposed to *Stachybotrys*.

42. Karoly ED, Li Z, Dailey LA, Hyseni X, Huang YCT. Up-regulation of tissue factor in human pulmonary artery endothelial cells after ultra-fine particle exposure. Environmental Health Perspectives 2007; 115(4): 535-540.

This paper discusses increased production by vascular endothelial cells in lung following exposure to inhaled inflammatory elements. The results of inhalation of particulate materials may cause adverse cardiovascular health effects.

43. Bird L. Regulatory circuit in the lungs. Nature Reviews Immunology 2006; 6: 426.

Alveolar macrophage activation can be inhibited by TGF β-1 released following exposure to microbial products.

44. Pestka JJ, Yike I, Dearborn DG, Ward MDW, Harkema JR. *Stachybotrys chartarum*, trichothecene mycotoxins and damp building-related illness: new insights into a public health enigma. Toxicological Sciences 2008; 104: 4-26.

This review article enumerates the biologically active components of WDB that can contribute to pathophysiologic effects from WDB.

45. Francis K, Lewis BM, Akatsu H, Monk PN, Cain SA, Scanlon MF, Morgan BP, Ham J, Gasque P. Complement C3a receptors in the pituitary gland: a novel pathway by which

an innate immune molecule releases hormones involved in the control of inflammation. FASEB J 2003; 17: 2266-2268.

Complement innate immune molecules and cytokines modulate tissue specific and systemic inflammatory responses through communication with the endocrine system.

46. Takafugi S, Ishida A, Miyakuni T, Nakagawa T. Matrix metalloproteinase-9 release from human leukocytes. J Investig Aller Clin Immunol 2003; 13: 50-5.

MMP9 is released from neutrophils following exposure to complement and cytokines.

47. Munford RS. Sensing gram-negative bacterial lipopolysaccharides: a human disease determinant? Infection Immunity 2008; 76: 454-465.

Recognition of lipopolysaccharides is critical to host defense. Harmful systemic inflammatory responses when LPS is sensed result in with cytokine release following exposure.

48. Griffiths M, Neal JW, Gasque P. Innate immunity and protective neuroinflammation: new emphasis on the role of neuroimmune regulatory proteins. Int Rev Neurobiol 2007; 82: 29-55.

In some diseases, protective innate immune mechanisms lead to neurodegeneration on the basis that several innate immune molecules have neurocytotoxic activities.

49. Holt PG, Strickland DH, Wikstrom ME, Jahnsen FL. Regulation of immunological homeostasis in the respiratory tract. Immunology 2008; 8: 142-152.

Important recent review of immune effectors in the respiratory system with a rich endowment of control and attack mechanisms in the 70 cubic meters of mucus membrane tissue in the lung. TGF β-1, pattern receptors, inflammatory mediators, antigen presenting cells and "all of innate immunity" meet here.

50. Bretz C, Gersuk G, Knoblaugh S, Chaudhary N, Randolph-Habecker J, Hackman RC, Staab J, Marr KA. MyD88 signaling contributes to early pulmonary responses to *Aspergillus fumigatus.* Infection Immunity 2008; 76: 952-958.

Macrophage inflammatory mechanisms are dependent on pattern receptor detection and subsequent immune signaling in lung following exposure to *A. fumigatus*.

51. Ramirez-Ortiz ZG, Specht CA, Wang JP, Lee CK, Bartholomeu DC, Gazzinelli RT, Levitz SM. Toll-like receptor 9-dependent immune activation by unmethylated CpG motifs in Aspergillus fumigatus DNA. Infection Immunity 2008; 76: 2123-2129.

Toll receptor 9 intracellularly detects *A. fumigatus*, resulting in secretion of pro-inflammatory cytokines contributing to the immune response to the pathogen.

52. DiScipio R, Schraufstatter I, Sikora L, Zuraw B, Sriramarao P. C5a mediates secretion and activation of matrix metalloproteinase 9 from human eosinophils and neutrophils. Int Immunopharmacol 2006; 7: 1109-18.

A split product of complement activation, C5a, brings about secretion of MMP9 by eosinophils and neutrophils.

53. Spirig R, van Kooten C, Obregon C, Nicod L, Daha M, Rieben R. The complement inhibitor low molecular weight dextran sulfate prevents TLRA 4-induced phenotypic and functional maturation of human dendritic cells. J Immunol. 2008;181:878-90.

Blocking complement adherence to antigen presenting cells prevents innate immune receptors, including TLR-4, from causing maturation of dendritic cells.

54. Koneti A, Linke MJ, Brummer E, Stevens DA. Evasion of innate immune responses: evidence for mannose binding lectin inhibition of tumor necrosis factor alpha production by macrophages in response to *Blastomyces dermatitidis*. Infection Immunity 2008; 76: 994-1002.

The mechanism of inhibition of innate immune inflammatory responses of macrophages to *Blastomyces* is mediated by serum MBL binding to (1-3)-β glucan sites thereby preventing stimulation of macrophages for TNF production.

55. Johanning E, Biagini R, Hull D. Health and immunology study following exposure to toxigenic fungi (*Stachybotrys chartarum*) in a water-damaged office environment. Int Arch Occup Environ Health 1996; 68: 207-218.

A case control study of 53 cases and 21 controls. Strong associations between illness symptoms of respiratory symptoms, dermatologic symptoms, chronic fatigue, constitutional symptoms and visual symptoms were found. Prolonged and intense exposure to toxigenic fungi are associated with respiratory and CNS findings.

56. Mazur L, Kim J. Spectrum of noninfectious health effects from molds. Pediatrics 2006; 118: 1909-1926.

There is evidence of fungal related diseases from exposure to WDB.

57. Santilli J. Health effects of mold exposure in public schools. Current Allergy and Asthma Reports 2002; 2: 460-467.

Mold exposure resulting from moisture damage has run rampant and is becoming a serious issue in school-age children. The paper reports rosters of symptoms including fatigue and many others.

58. Brasel T, Campbell A, Demers R, Ferguson B, Fink J, Vojdani A, Wilson S, Straus D. Detection of trichothecene mycotoxins in sera from individuals exposed to *Stachybotrys chartarum* in indoor environments. Arch Environ Health 2004; 59: 317-23.

Using ELISA, trichothecene mycotoxins can be demonstrated in the sera of individuals exposed to S. chartarum in contaminated buildings.

59. Hooper D, Bolton V, Guilford F, Straus D. Mycotoxin detection in human samples from patients exposed to environmental molds. Int. J. Mol. Sci. 2009; 10: 1465-1475.

Antibody testing can identify toxins accurately in human tissues and body fluids following exposure to WDB.

60. Hymery N, Leon K, Carpentier F, Jung J, Parent-Massin D. T-2 toxin inhibits the differentiation of human monocytes into dendritic cells and macrophages. Toxicol In Vitro 2009; 3: 509-19.

T-2 toxin disturbs human monocyte differentiation processes. These results could significantly contribute to immunosuppressive properties.

61. Hymery N, Sibiril Y, Parent-Massin D. In vitro effects of trichothecenes on human dendritic cells. Toxicol in Vitro 2006; 6: 899-909.

Trichothecenes have adverse effects on dendritic cells and dendritic maturation process.

62. Pei L, Shan S, Sun X. Effects of injuries pulmonary arterial endothelial cell induced by endotoxin on proliferation of pulmonary artery smooth muscle cells and interference effect of bone morphogenetic protein-2. Zhonghua Yi Xue Za Zhi 2008; 1: 40-5.

Endotoxins injure pulmonary artery endothelial cells, smooth muscle cells and alter gene activation.

63. Mastronardi C, Srivastava V, Yu W, Les Dees W, McCann S. Lipopolysaccharide-induced leptin synthesis and release are differentially controlled by alpha-melanocyte-stimulating hormone. Neuroimmunomodulation 2005; 3: 182-8.

MSH inhibits synthesis and release of pro-inflammatory cytokines.

64. Dubourdeau M, Athman R, Ballov V, Huerre M, Chignard M, Philpott D, Latge J, Ibrahim-Granet O. *Aspergillus fumigatus* induces innate immune responses in alveolar macrophages through the MAPK pathway independently of TLR2 and TLR4. J Immunol 2006; 6: 3994-4001.

The signaling pathways of alveolar macrophages following exposure to *Aspergillus* are not well understood. Toll signaling with MyD88 and ERK activation are important in innate immunity to *Aspergillus.*

65. Brzoska T, Luger T, Maaser C, Abels C, Bohm M. Melanocyte-stimulating hormone and related tripeptides: Biochemistry, anti-inflammatory and protective effects in vitro and in vivo, and future perspectives for the treatment of immune-mediated inflammatory diseases. Endocrine Reviews 2008; 5: 581-602.

MSH is anti-inflammatory and protective for cells of the immune system and non-immune cells. It is suitable for use in treatment of inflammatory processes.

66. Hogaboam C, Takahashi K, Ezekowitz R, Kunkel S, Schuh J. Mannose-binding lectin deficiency alters the development of fungal asthma: effects on airway response, inflammation, and cytokine profile. J Leukoc Biol 2004; 5: 805-14.

MBL participates in pulmonary innate immune responses to *A. fumigatus*.

67. Siddiqui AA, Shah AA, Bashir SJ. Craniocerebral aspergillosis of sino-nasal origin in immuno-competent patients: Clinical Spectrum and outcome of 25 cases. Neurosurgery 2004; 55:602-13.

This paper presents the clinical outcome of craniocerebral aspergillosis arising from sino-nasal involvement. The types of craniocerebral involvement are: Type I-Intracerebral aspergillosis (66.7 mortality rate); Type II-intracranial extra-dural aspergillosis; and Type III-cranial base aspergillosis. Presenting symptoms included: nasal stuffiness, headaches, nasal discharges, proptosis. All patients had surgical intervention and oral intraconazole. Paranasal sinus infections can lead to craniocerebral aspergillosis in immunocompetent individuals.

Mechanisms of Illness Acquisition

Please review some of the references that insist that inhalation, not ingestion, is the source of exposure that leads to illness.

Governmental agency opinions:

Centers for Disease Control and Prevention:

1. Chew GL, Wilson J, Rabito FA, Grimdley F, Iqbal S, Reponen T, Muilenberg ML, Thorne PS, Dearborn DG, Morley RL. Mold and endotoxin levels in the aftermath of hurricane Katrina: a pilot of homes in New Orleans undergoing renovation. Env Health Perspectives 2006; 114: 1883-1889.

This 2006 paper looks at homes in New Orleans flooded for weeks following Hurricane Katrina. Spore counts at baseline range from 22,000 to 515,000 cfu and endotoxin range from 17 to139 units/m3. Use of respirators and protection such as elastomerics is suggested to prevent acquisition of illness prospectively.

2. Redd SC. State of the science on molds and human health. 2002; 1-10.

This address to a House sub-committee in the US House of Representatives provided a brief overview of the CDC opinions in 2002 regarding health risk from mold exposure. This paper is included simply to show the dramatic shift in CDC opinion since 2002 to the Rao paper cited below.

3. Rao CY, Riggs MA, Chew GL, Muilenburg ML, Thorne PS, Van Sickle D, Dunn KH, Brown C. Characterization of airborne molds, endotoxins, and glucans in homes in New Orleans after Hurricanes Katrina and Rita. Applied and Environmental Microbiology 2007; 73(5): 1630-1634.

This paper, written by some of the same authors as paper #1 in this section confirms that the levels of endotoxins, beta-glucans and molds that were detected in environments after Hurricane Katrina and Rita were found at concentrations that are associated with health effects. Species and concentrations were different from those previously reported from non-water damaged buildings in the Southeast. Moreover, in this study the authors expressed gratitude to the Office of the Inspector General for insuring the safety of the sampling teams. This paper signals the 180° shift of CDC opinion.

4. Verhoeff AP, Burge HA. Health risk assessment of fungi in home environments. Ann Allergy Asthma Immunol 1997; 78: 544-556.

This older paper (published 1997) confirms that one or more positive associations were found between fungal levels and health outcomes in seven of nine cross sectional studies. Curiously, the paper concludes that it is impossible to set guidelines for fungi and home environments based on health risk assessment. Future research designed to generate data that can be used for the development of risk assessment should focus on susceptible

populations and use measures that accurately represent exposure and adverse health effects.

5. Gent JF, Ren P, Belanger K, Triche E, Bracken MB, Holford TR, Leaderer BP. Levels of household mold associated with respiratory symptoms in the first year of life in a cohort at risk for asthma. Environmental Health Perspectives 2002; 110(12): A781-A786.

This paper published in 2002 is quoted by Rao in the 2007 CDC paper. It confirms a high risk of association of illness following exposure to moldy environments.

6. Solomon GM, Hjelmroos-Koski M, Rotkin-Ellman M, Hammond SK. Airborne mold and endotoxin concentrations in New Orleans, Louisiana, after flooding, October through November 2005. Environmental Health Perspectives 2006; 114(9): 1381-1386.

Looking at airborne mold and endotoxin concentrations in New Orleans, these authors conclude that high concentrations of mold measured indoors and outdoors in flooded areas are likely to be a significant respiratory hazard that should be monitored over time. Workers and returning residents should use appropriate and protective equipment and exposure mitigation techniques to prevent respiratory morbidity in long-term health effects (NOTE: this paper does not include prospective assessment of change of health status following exposure and provides no mechanism of injury to exposed patients even though those data were readily available to the CDC in 2005 and 2006). This opinion paper from the CDC on Mold Prevention Strategies published in 2005 is part of the "old line" approach of the CDC ignoring confirmed health effects.

7. Brandt M, Burkhart J, Burton NC, Cox-Ganser J, Damon S, Falk H, Fridkin S, Garbe P, Kreiss K, McGeehin M, Morgan J, Page E, Rao C, Redd S, Sinks T, Trout D, Wallingford KM, Warnock D, Weissman DN. Mold Prevention strategies and possible health effects in the aftermath of Hurricanes Katrina and Rita. CDC October 2005; 1-45.

This is the opinion of the CDC mold group published in October 2005. The damage from hurricanes Katrina and Rita occurred in September 2005. There are several chapters looking at illness acquired following exposure to water-damaged buildings. This statement does not acknowledge adequate documentation of health effects from inhalation exposures to toxins but "given the lack of information regarding non-ingestion mycotoxin exposure and adverse health effects in humans, it would be prudent to take precautions when handling heavily contaminated building materials." Contrast this statement advising "prudence" to the statement from March 2007 confirming associations of human illness with exposure to the very same mycotoxins, beta-glucans and endotoxins the CDC reviewed in 2005. Passage of two years did not change the behavior of these compounds; it changed the behavior of the government agency.

8. Dangman KH, Bracker AL, Storey E. Work-related asthma in teachers in Connecticut: Association with chronic water damage and fungal growth in schools. Conn Med 2005; 69:9-16.

In this paper the authors reviewed the respiratory health of teachers in WDB and moisture free buildings. WDB teachers had incidence of 75 % upper respiratory symptoms versus 45 % in dry buildings. Twenty-two of 55 teachers had rhinitis/sinusitis; 23 had asthma; and four had cases of granulomatous disease (two hypersensitivity pneumonitis and two with sarcoidosis). The authors concluded: "Work-place exposures in water-damaged school buildings are risk factors for development of lower respiratory disease in school teachers and staff."

The Canada Ministry of Health

9. Minister of Health. Residential indoor air quality guideline for moulds. 2007-03-31 Canada Gazette Part I, 141(13).

The Minister of Health recommends controlling humidity intelligently, repairing any water damage in residences involving mold growth and cleaning any visible or concealed mold growing in any residential buildings. These recommendations apply regardless of the mold species found to be growing in the building. Conclusion: the official Residential Indoor Air Quality Guidelines from Health Canada considers that mold growth in residential buildings poses a health hazard. Health risk depends on exposure and on allergic sensitization. A large number of mold species and strains growing in buildings and the large inter-individual variability in human response to mold exposure precluded the derivation of exposure limitants. Therefore, Health Canada recommends that mold be cleaned up to prevent human health effects and that in the absence of exposure limits results from tests for the presence of fungi air cannot be used to assess risk to the health of building occupants.

EPA

10. Mendell MJ, Cozen M, Lei-Gomez Q, Brightman HS, Erdmann CA, Girman JR, Womble SE. Indicators of moisture and ventilation system contamination in U.S. office buildings as risk factors for respiratory and mucous membrane symptoms: analyses of the EPA BASE data. J Occup Environ Hyg 2006; 3(5): 225-233.

This paper looks at association of lower respiratory symptoms and moisture measures in buildings. There is a three-fold increase in symptoms associated with a lack of cleaning drip pans under air-conditioning coils.

11. Mudarri D, Fisk WJ. Public health and economic impact of dampness and mold. Indoor Air 2007; 17: 226-236.

This is a landmark study showing that the national annual cost of asthma that is attributed to mold exposure in the home is estimated to be $3.5 billion. This analysis indicates an exposure to dampness and mold in buildings poses both significant public health and economic risk. Of 21.8 million people reported to have asthma in the United States, 4.6 million cases are estimated to be attributed to dampness or mold exposure in the home. (NOTE this study does not take into account water-damaged schools and water-damaged

office buildings. The numbers are much higher when the additional risk from exposure outside the home is factored in.)

NIEHS

12. Weinhold B. A spreading concern on inhalational health effects of mold. Environmental Health Perspectives 2007; 115(6): A300-305.

In this lead editorial for a mini-monograph, Weinhold reviews a variety of factors possibly contributing to the undisputed increase in health effects related to exposure to moisture in water-damaged buildings. He reviews a number of papers in this summary of references including Drs. Fisk, Huttunen, Pestka, and Vesper. He notes that the conclusions of Health Canada as the Minister of Health in Canada determined that any visible indoor mold poses a health hazard. Weinhold discusses the EPA guidelines for moisture control practices. His final conclusion is quite clear: *"Don't mess with mold. If you can see it or smell it, and especially if health problems are occurring, clean it out, throw it out or get out."* He notes the skepticism of ACOEM and ACMT persists.

13. Wu F, Jacobs D, Mitchell C, Miller D, Karol MH. Improving indoor environmental quality for public health: impediments and policy recommendations. Environmental Health Perspectives 2007; 115(6): 953-957.

This overview of potential sources of exposure emphasizes the importance of moisture in studies worldwide looking at health effects. A questionnaire of homes of 6,273 children revealed up to 58% of the homeowners reported water in the basement and water damage to the building or mold on any surface in the homes. In 274 homes in Seattle, Washington where patients with asthma resided 44% contained visible mold. In a study of 1,600 homes throughout Europe, Australia, India, New Zealand and the United States, 22% of the homeowners reported mold or mildew problems within the last year of their time at home. She concludes that public health education and information about healthier homes is a public policy that is supported by academic literature.

14. Mitchell CS, Zhang J, Sigsgaard T, Jantunen M, Lioy PJ, Samson R, Karol MH. Current state of the science: health effects and indoor environmental quality. Environmental Health Perspectives 2007; 115(6): 958-964.

Mitchell discusses a variety of potential sources of human illness that are pollutants in indoor environments. He quotes a study (Meyer 2005) showing a statistically significant increase in mucus membrane symptoms following exposure in a double blinded study of 8 sensitive school employees to different kinds of molds totaling six minutes on three separate days. He cites Shoemaker and House 2005 as a study involving treatment. He concludes that it is increasingly apparent that indoor environments can provide significant exposures that affect the health of occupants.

15. Loftness V, Hakkinen B, Adan O, Nevalainen A. Elements that contribute to healthy building design. Environmental Health Perspectives 2007; 115(6): 965-970.

Discusses many variables associated with developing healthy homes including use of fungal-resistant construction materials and finishes.

16. Wu F, Takaro TK. Childhood asthma and environmental interventions. Environmental Health Perspectives 2007; 115(6): 971-975.

This paper emphasizes the importance of education to reduce illness triggers inside a home including mechanical means such as HEPA and simple heating systems that reduce dampness and reduce particulates in indoor air, which in turn improve asthma indicators.

17. Jacobs DE, Kelly T, Sobolewski J. Linking public health, housing and indoor environmental policy: successes and challenges at local and federal agencies in the United States. Environmental Health Perspectives 2007; 115(6): 976-981.

This paper reviews public health policy regarding interior home environments beginning with a history of realization that the home environment presents significant contributors to health effects. The study confirms that inadequate ventilation or moisture management in housing still contributes to asthma, mold-induced illnesses and other injuries. The study discusses that the most significant risk factor was a forced air furnace design in which all but the supply air was drawn from the basement space and none from the living space. This resulted in the migration of significant volumes of air from the basement space to the occupied areas of the housing unit. Asthma triggers, irritants, pathogens and other exposure risks that collect in damp basement air were then distributed into breathing air in living spaces. Basements often have the highest incidence of water infiltration, water damage and mold growth of any other room in the housing structure.

18. Adan OCG, Ng-A-Tham J, Hanke W, Sigsgaard T, van de Hazel P, Wu F. In search of a common European approach to a healthy indoor environment. Environmental Health Perspectives 2007; 115(6): 983-988.

This paper quotes the relationship between housing and health in the WHO/Pan-European survey. Moisture and mold were found in 25% of dwellings. An additional 8% of dwellings had smells and dampness that were “determined links” to respiratory disease, asthma, allergies and fatigue.

See also Damp Indoor Spaces and Health, Institute of Medicine, 2004; the American Indoor Hygiene Association (Green Book 2008); the Government Accountability Office report of 9/09; the World Health Organization (May 2008 newsletter and July 2009 report). Ingestion is never the problem. Inhalation is the mechanism of delivery of toxigens and inflammagens into the host.

NIOSH has a number of papers supporting inhalation, including:

19. Park JH, Cox-Ganser JM, Kreiss K, White SK, Rao CY. Hydrophilic fungi and ergosterol associated with respiratory illness in a water-damaged building. Environmental Health Perspectives 2008; 116(1): 45-50.

Measurements of ergosterol as a measure of fungal body mass shows promise as markers of building-related respiratory diseases in damp indoor environments.

20. Park JH, Schleiff PL, Attfield MD, Cox-Ganser JM, Kreiss K. Building-related respiratory symptoms can be predicted with semi-quantitative indices of exposure to dampness and mold. Indoor Air 2004; 14: 425-433.

This paper from NIOSH has been included in three separate sections; comments about non-linear dose response effects precede this paper from 2006. Specifically, there are clear relationships from exposure to response between exposure to moldy buildings and health effects. This paper was confined to work exposure and adds significantly to government opinion regarding safety of mold exposure. This study confirms that based solely on visual and olfactory observations, and accounting for time spent in specific rooms, one can predict existence of excessive building-related symptoms and diseases. From a public health perspective, these observations justify immediate action to correct water leaks and repair water damage in order to prevent building-related respiratory diseases. This paper does not demand isolation of individual fungi or mycotoxins to conclude that the illness was caused by exposure.

21. Park JH, Cox-Ganser J, Rao C, Kreiss K. Fungal and endotoxin measurements in dust associated with respiratory symptoms in a water-damaged office building. Indoor Air 2006; 16(3): 192-203.

This paper has been referenced previously; the importance of this NIOSH study is to show non-linear relationships between exposure to toxigenic elements and health effects.

22. Rao CY, Cox-Ganser JM, Chew GL, Doekes G, White S. Use of surrogate markers of biological agents in air and settled dust samples to evaluate a water-damaged hospital. Indoor Air 2005; 15 Suppl 9: 89-97.

This is another NIOSH study in which the authors measured cultured fungi and bacteria; fungal spores; and endotoxin in sub-micron particles and air; as well as cultured fungi, bacterial endotoxins, extracellular polysaccharides, ergosterol and (1-3)-β-glucans in chair and floor dust. They found that sub-micron particles and markers of microbiological agents, but not cultured microbiological agents, were significantly positively associated with buildings with water damage and higher prevalence of reported respiratory symptoms. They concluded that detection and quantification of non-culture based microbiologic markers may be useful methods to assess microbial contamination and indeed more accurately evaluate microbial exposure in indoor environments.

23. Cox-Ganser JM, White SK, Jones R, Hilsbos K, Storey E, Enright PL, Rao CY, Kreiss K. Respiratory morbidity in office workers in a water-damaged building. Environmental Health Perspectives 2005; 113(4): 485-490.

This is another in a series of papers from NIOSH looking at building-related respiratory disease in offices with water intrusion in Northeastern United States. 888 individuals showed an increased prevalence in respiratory health symptoms with exposure to water-damaged buildings compared to those without such exposure as well as compared to

those with exposure, but then subsequently kept away from the building. Occupancy of the water-damaged building was associated with the onset and exacerbation of respiratory conditions, confirmed by objective medical tests.

24. Sorenson WG. Fungal spores: hazardous to health? Environmental Health Perspectives 1999; 107(3): 469-472.

This 1999 NIOSH overview of illness caused by exposure to fungi acknowledges presence of mycotoxins in spores, effects of mycotoxins on immune macrophages and immune functions, and the risk of illness following respiratory inhalation. Sorenson notes that T-2 toxins, when given by inhalation, were at least 10 times more toxic than systemic administration and at least twenty times more toxic than dermal administration.

Academic institutions have reported on inhalation as the mechanism of exposure.

Pertinent papers include:

25. Bernstein JA, Alexis N, Bacchus H, Bernstein IL, Fritz P, Horner E, Li N, Mason S, Nel A, Oullette J, Reijula K, Reponen T, Seltzer J, Smith A, Tarlo SM. The health effects of non-industrial indoor air pollution. J Allergy Clin Immunol 2008; 1-7.

This "quick overview" paper looks at a variety of possible indoor pollutants; it contains only a few paragraphs on microbial contamination. The authors confirm that numerous studies conducted worldwide have reported an association between damp or mold and adverse health effects. Moisture and microbes in buildings can affect human health by a variety of biological mechanisms including infections, allergic or hypersensitivity reactions and irritant reactions. Toxins contribute to the illness. Ultra-fine and nano-size particles called fragments carry the bulk of allergens, mycotoxins and beta-glucans.

26. Ammann HM. Is indoor mold contamination a threat to health? Washington State Dept. of Health.

This clearly-stated summary of the state of knowledge of indoor mold exposure in 2004 is discussed by Dr. Harriett Ammann. She was a member of the IOM panel; she wrote the chapter on Toxicology and added to the chapter on Public Health Concerns. She discusses the importance of peer-reviewed literature on the study of acquisition of human health effects following exposure to water-damaged buildings.

27. Chao HJ, Schwartz J, Milton DK, Burge HA. Populations and determinants of airborne fungi in large office buildings. Environmental Health Perspectives 110(8): 777-782.

This paper from Dr. Burge's group in Harvard sampled airborne fungal populations in office buildings without evidence of water intrusion or microbial amplification. There is a seasonal variation of airborne fungal populations.

28. Wilson SC, Carriker CG, Brasel TL, Karunasens E, Douglas DR, Wu C, Andriychuk LA, Fogle MR, Martin JM, Straus DC. Culturability and toxicity of sick building

syndrome-related fungi over time. Journal of Occupational and Environmental Hygiene 2004; 1: 500-504.

Toxigenic fungi can be cultured from swabs of samples taken from buildings with water intrusion that have since been dry following prolonged storage from up to 266 days. There is no loss of toxicity with such storage.

29. Meyer HW, Jensen KA, Nielsen KF, Kildeso J, Norn S, Permin H, Poulsen LK, Double-blinded, placebo-controlled exposure to molds: exposure system and clinical results. Indoor Air 2005; Suppl 10: 73-80.

8 patients with a history of skin test allergy to *Penicillium* were exposed to either placebo or mold spores for six minutes each on three separate days. There was no difference in response of these patients to spores. This study is misleading for many reasons, including absence of control group, absence of use of objective measures of laboratory changes, absence of a thorough questionnaire, absence of validation of a questionnaire and absence of exposure to the entirety of elements found in water-damaged buildings. This study suggests that there is no change in allergy in those with a history of allergy who breathe mold spores for short periods of time i.e. for three days.

30. Kildeso J, Wurtz H, Nielsen KF, Kruse P, Wilkins K, Thrane U, Gravesen S, Nielsen PA, Schneider T. Determination of fungi spore release from wet building materials. Indoor Air 2003; 13(2): 148-155.

This paper demonstrates that spores growing on gypsum board are released following air disturbance. Some of these particles released are much smaller than spore size.

31. Sivasubramani SK, Niemeier RT, Reponen T, Grinshpun SA. Assessment of the aerosolization potential for fungal spores in moldy homes. Indoor Air 2004; 14(6): 405-412.

This paper from the Cincinnati group looks at the errors inherent in air testing. The authors suggest that an additional mechanism be used to calculate potential health risk.

32. MacIntosh DL, Brightman HS, Baker BJ, Myatt TA, Stewart JH, McCarthy JF. Airborne fungal spores in a cross-sectional study of office buildings. J Occup Environ Hyg 2006; 3(7): 379-389.

The results from this study contrast with the Burge paper (#3 in this section) presented previously; in this study airborne fungal spores were found to show little variation between time of day and season over the year.

33. Kuhn RC, Trimble MW, Hofer V, Lee M, Nassof RS. Prevalence and airborne spore levels of Stachybotrys spp. in 200 houses with water incursions in Houston, Texas. Can J Microbiol 2005; 51(1): 25-28.

In 200 homes with water intrusion *Stachybotrys* was found in 58.5%. Less than 10% of air samples contained *Stachybotrys*. Aerosolizaton of *Stachybotrys* correlated with both

wall cavity and surface contamination, though the contribution from wall cavities was low.

34. Barnes CS, Dowling P, Van Osdol T, Portnoy J. Comparison of indoor fungal spore levels before and after professional home remediation. Ann Allergy Asthma Immunol 2007; 98(3): 262-268.

This paper looks at spore counts before and after remediation in water-damaged buildings. 88% had Aspergillus/Penicillium; *Stachybotrys* was present in 53%. Remediation reduced spore counts from 131,000 to 1,300; there is no discussion of any other elements commonly found in water-damaged buildings reported in the study. Many experts feel that spore counts of 1,300 in respirable air are too high.

35. Khan NN, Wilson BL. An environmental assessment of mold concentrations and potential mycotoxin exposures in the greater Southeast Texas area. J Environ Sci Health A Tox Hazard Subst Environ Eng 2003; 38(12): 2759-2772.

Following Hurricane Allison, testing of homes over a three months period in the Houston, Texas area showed that *Aspergillus, Penicillium, Chaetomium*, and *Stachybotrys* were found in tape lifts in bulk assays.

36. Santilli J, Rockwell W. Fungal contamination of elementary schools: a new environmental hazard. Ann Allergy Asthma Immunol 2003; 90(2): 203-208.

Fungal testing should be done in schools with a history of water-damage ensure reduction of spore counts to less than 1000 spores/m3.

37. Savilahti R, Uitti J, Laippala P, Husman T, Roto P. Respiratory morbidity among children following renovation of a water-damaged school. Arch Environ Health 2000; 55(6): 405-410.

Moisture damage and exposure to molds increase indoor air problems in schools and affect the respiratory health of children.

38. Kercsmar CM, Dearborn DG, Schluchter M, Xue L, Kirchner HL, Sobolewski J, Greenberg SJ, Vesper SJ, Allan T. Reduction in asthma morbidity in children as a result of home remediation aimed at moisture sources. Env Health Perspectives 2006; 114: 1574-1580.

Remediation of water-damaged buildings reduces moisture sources and significantly reduces symptom days and health care use for asthmatic children who lived in the moldy homes.

39. Horner, Elliott W. The damp building effect: understanding needed not more debate. Ann of Allergy, Asthma & Immunology 2005; 94: 213-215. Abstract not available.

40. Shenassa ED, Daskalakis C, Liebhaber A, Braubach M, Brown MJ. Dampness and mold in the home and depression: an examination of mold-related illness and perceived

control of one's home as possible depression pathways. American Journal of Public Health 2007; 97(10): 1-7.

This 2007 paper confirmed the association of exposure to water-damaged buildings with psychiatric symptoms including depression. This is based on survey data from 8 European cities. There are no biomarkers to support the physical basis of psychiatric symptoms.

41. Hope AP, Simon RA. Excess dampness and mold growth in homes: an evidence-based review of the aero-irritant effect and its potential causes. Allergy Asthma Proc 2007; 28(3): 262-270.

This paper from AAAAI states plainly that exposure to fungi produces respiratory diseases in humans through allergic and non-allergic mechanisms. Further, the preponderance of epidemiologic data supports the link between exposure to dampness and excessive mold growth in the development of aero-irritant symptoms. These studies support the role of VOCs contributing to symptoms in occupants of damp and mold-contaminated homes.

42. Bornehag CG, Blomquist G, Gyntelberg F, Jarvholm B, Malmberg P, Nordvall L, Nielsen A, Pershagen G, Sundell J. Dampness in buildings and health. Nordic interdisciplinary review of the scientific evidence on associations between exposure to "dampness" in buildings and health effects (NORDDAMP). Indoor Air 2001; 11(2): 72-86.

This paper reports a literature search identifying 590 peer-reviewed articles that deal with damp buildings, sixty one of which are referenced that show a significant increase of relative risk of health effects from water-damaged buildings from 1.4 to 2.2. The study also reports the association between dampness and symptoms such as tiredness, headache and airways infection. This study supports a hypothesis that health problems can result from exposure to dampness and fungi in indoor air. The authors conclude that such exposure is unacceptable from a public health perspective.

43. King N, Auger P. Indoor air quality, fungi and health. How do we stand? Can Fam Physician 2002; 48: 298-302.

This 2002 paper from Montreal concludes that the various health problems that can result from dampness and fungi in indoor air make such exposure unacceptable from a public health perspective.

44. Hodgson MJ, Morey P, Leung WY, Morrow L, Miller D, Jarvis BB, Robins H, Halsey JF, Storey. Building-associated pulmonary disease from exposure to *Stachybotrys chartarum* and *Aspergillus versicolor*. J Occup Environ Med 1998; 40(3): 241-249.

This paper describes the illness symptoms and mold contamination of the Martin County Courthouse alongside the St. Lucie River on the Atlantic coast of Florida. The authors report that the 3-15 fold increase in symptoms represents "a likely human response to inhaled fungal toxins in indoor environments."

45. Reijula K, Tuomi T. Mycotoxins of aspergilli: exposure and health effects. Front Biosci 2003; 8: s232-235.
This is a review of general health effects from exposure to *Aspergillus* mycotoxins.

46. Horner WE, Worthan AG, Morey PR. Air- and dust mycoflora in houses free of water damage and fungal growth. Appl Environ Microbiol 2004; 70: 6394-6400.

Looking at fungal contamination in non water-damaged buildings, the authors did not find differences between indoor and outdoor fungi in control buildings.

47. Murtoniemi T, Nevalainen A, Suutari M, Toivola M, Komulainen H, Hirvonen MR. Induction of cytotoxicity and production of inflammatory mediators in raw 264.7 macrophages by spores grown on six different plasterboards. Inhal Toxicol 2001; 13(3): 233-247.

This paper shows that spores taken from fungi growing on six different kinds of plasterboard stimulated production of inflammatory compounds including nitric oxide, IL-1β and other interleukins. The data indicated clear differences between spores of different microbes and the ability to induce production of these inflammatory mediators and to cause cell death. Interestingly, the spores from the actinomycetes organisms were the most potent at inducing cytokine production as well as causing direct cell death.

48. Bloom E, Bal K, Nyman E, Must A, Larsson L. Mass spectrometry-based strategy for direct detection and quantification of some mycotoxins produced by *Stachybotrys* and *Aspergillus* spp. in indoor environments. Applied and Environmental Microbiology 2007; 73(13): 4211-4217.

This is another paper looking at whether or not mycotoxins are found in water-damaged buildings. The authors use mass spectroscopy to confirm that mycotoxins produced by *Aspergillus* and *Stachybotrys* are found in buildings with ongoing dampness and a history of water damage. The direct detection of highly toxic sterigmatocystin and macrocyclic trichothecene mycotoxins in indoor environments is important to their potential health impacts.

49. Boutin-Forzano S, Charpin-Kadouch C, Chabbi S, Bennedjai N, Dumon H, Charpin D. Wall relative humidity: a simple and reliable index for predicting *Stachybotrys chartarum* infestation in dwellings. Indoor Air 2004; 14(3): 196-199.

This paper looks at health effects from a different perspective using relative wall humidity as a factor to associate with growth of *Stachybotrys*. There was no straightforward relationship between the relative humidity of the wall and in the room but the wall relative humidity itself was a reliable index for evaluating the potential for *Stachybotrys* infestation.

50. Menzies D, Comtois P, Pasztor J, Nunes F, Hanley JA. Aeroallergens and work-related respiratory symptoms among office workers. J Allergy Clin Immunol 1998; 101(1): 38-44.

This is an allergy paper showing that in Montreal, Canada there is an association between exposure to molds in indoor environments and development of allergic findings, including positive skin tests.

51. Meyer HW, Wurtz H, Suadicani P, Valbjern O, Sigsgaard T, Gyntelberg F. Molds in floor dust and building-related symptoms among adolescent school children: a problem for boys only? Indoor Air 2005; 15(Suppl 10): 17-24.

This paper looked at 1024 adolescent children with exposure to either eight wet or seven dry buildings. There was an increase in symptoms from the wet buildings, more commonly seen in boys compared to girls, but significantly increased in the wet buildings compared to dry buildings.

52. Bornehag G, Sundell J, Sigsgaard T. Dampness in buildings and health (DBH): report from an ongoing epidemiological investigation on the association between indoor environmental factors and health effects among children in Sweden. Indoor Air 2004; 14(Suppl 7): 59-66.

This study was published in 2004 from work done on 14,077 school children in the year 2000. The study looked carefully at possible sources of biases when evaluating building exposures and found none. The self-reported moisture-related problems in buildings were associated with asthma, allergy and airway problems in children and adults. They validated their questionnaire and identified risk factors for symptoms which confirmed dampness, observed by a third party, low ventilation rate, presence of endotoxin and presence of *Penicillium* species in dust.

53. Andersson MA, Nikulin M, Koljajg U, Andersson MC, Rainey F, Reijula K, Hintikka EL, Salkinojia-Salonen M. Bacteria, molds, and toxins in water-damaged building materials. Applied and Environmental Microbiology 1997; 63(2): 387-393.

This paper discusses the shortcomings of various techniques used in the 90's to evaluate the importance of gram negative endotoxins, (1-3)-β-glucans and mycotoxins. A cell system using boar spermatozoa effectively showed toxicity. The absence of use of this test in the 90's and early 2000's contributed to lack of understanding of toxicity following exposure to water-damaged buildings.

54. Dales RE, Burnett R, Zwanenburg H. Adverse health effects among adults exposed to home dampness and molds. Am Rev Respir Dis 1991; 143: 505-509.

This 1990 study of 14,799 adults from six regions of Canada showed increased respiratory symptoms in patients reporting dampness or mold compared to those not reporting dampness or mold. There was a mild increase in symptoms of smokers compared to non-smokers. The odds ratio between symptoms and dampness was 1.62 in the final model taken (including smoking), that isolated damp buildings as a cause of illness without requiring documentation of presence of mycotoxins or given species of fungi.

55. Peltola J, Andersson MA, Haahtela T, Mussalo-Rauhamaa H, Rainey FA, Kroppenstedt RM, Samsom RA, Salkinoja-Salonen MS. Toxic-metabolite-producing

bacteria and fungus in an indoor environment. Applied and Environmental Microbiology 2001; 67(7): 3269-3274.

This paper comes from Dr. Andersson's lab (see reference 37). The boar spermatozoa model showed toxicity from actinomycetes as well as *Trichoderma* and endotoxins from bacterial isolates found in water-damaged buildings.

56. Page EH, Trout DB. The role of Stachybotrys mycotoxins in building-related illness. AIHAJ 2001; 62: 644-648.

Page and Trout reported in 2001 in a paper from NIOSH. They discussed the difficulty of exposure assessment in the absence of case definition for evaluating the role of mycotoxins as a cause of a building-related illness. They call for research to identify isolate-specific fungal toxins from the environment in humans before a more definitive link between health outcomes and mycotoxins can be made. They do not review any biomarkers that would substantiate their calls for research.

57. Mahooti-Brooks N, Storey E, Yang C, Simcox NJ, Turner W, Hodgson M. Characterization of mold and moisture indicators in the home. Journal of Occupational and Environmental Hygiene 2004; 1: 826-839.

This paper from Storey and Hodgson, the same authors of the Martin County Courthouse paper, review the potential for water damage in the homes of 64 patients. They find the basements with a musty odor, efflorescence, water intrusion or mold have a 2-3 fold increase in fungal concentrations over basements without these indicators. They showed also that basement water sources are important moisture mold indicators for epidemiologist to use to use in exposure assessment.

58. Gunnbjornsdottir MI, Franklin KA, Norback D, Bjornsson E, Gisason D, Lindberg E, Svanes C, Omenaas E, Norrman E, Jogi R, Jensen EJ, Dahlman-Hoglund A, Janson C. Prevalence and incidence of respiratory symptoms in relation to indoor dampness: the RHINE study. Thorax 2006; 61: 221-225.

This is a 2006 paper looking at 16,190 subjects from Iceland, Norway, Sweden, Denmark and Estonia. These subjects were previously evaluated in an earlier study. They found ongoing problems of increased prevalence of wheezing, breathlessness, cough and asthma for patients living in damp housing, even after statistical manipulation of potential confounders.

59. Jacob B, Ritz B, Gehring U, Koch A, Bischof W, Wichmann HE, Heinrich J. Indoor exposure to molds and allergic sensitization. Environmental Health Perspectives 2002; 110(7): 647-653.

This paper is from Germany looking at two separate studies done, one from 1992 and one from 1995 evaluating 2,470 patients. They find increased respiratory illness in children exposed to water-damaged buildings.

60. Gunnbjornsdottir MI, Norback D, Plaschke P, Norrman E, Bjornsson E, Janson C. The relationship between indicators of building dampness and respiratory health in young Swedish adults. Respir Med 2003; 94(4): 302-307.

This is an earlier paper looking at respiratory health effects in Swedish adults. There were 1,853 cases identified of whom over 205 reported water-damaged indoor mold growth during the study time. Comparing subjects with exposure to water-damaged buildings to those without such exposure showed an increased risk of illness symptoms in patients with exposure to water-damaged buildings.

61. Kyle AD, Woodruff TJ, Axelrad DA. Integrated assessment of environment and health: America's children and the environment. Environmental Health Perspectives 2006; 114(4): 447-452.

This is a cooperative EPA study looking at environmental illness in children. This paper needs to be compared to the 2007 EPA paper implicating mold as a factor causing asthma in 21% of all Americans. This academic paper looks at selected outdoor and indoor pollutants, drinking water contaminants, heavy metals and other factors but ignores water-damaged buildings completely.

62. Weschler CJ, Wells JR, Poppendieck D, Hubbard H, Pearce TA. Workgroup report: indoor chemistry and health. Environmental Health Perspectives 2006; 114(3): 442-504.

This is a summary of the findings of a group of experts convened to look at indoor chemistry and health in 2004. They develop a framework for finding exposures from indoor chemical entities and understanding a link between exposures to various health outcomes. One of the key foci of the workshop was to look at potential health effects relating association of inhalation exposure to products of indoor chemistry focusing on compounds released in water-damaged buildings. This paper produced several tested hypothesis though none have been funded by government agencies, now four years later.

63. Miller JD, Rand TG, Jarvis BB. *Stachybotrys chartarum*: cause of human disease or media darling? Medical Mycology 2003; 41: 271-291.

This 2003 paper discusses the literature on associations of illness following exposure to *Stachybotrys* in human and animals. The authors agreed that it is generally accepted that living and working in moldy environments is associated with building-related asthma, exacerbating asthma in mold susceptible asthmatics and increasing rates of upper respiratory disease. They do not find any evidence in 2003 in literature to account for neurologic damage resulting from exposure to mold. Compare this study to work from many other authors including Michigan State, Brown University and CRBAI, showing clear mechanisms of acquisition of neurologic injury following exposure to water-damaged buildings.

64. Jaakkola MS, Nordman H, Piipari R, Uitti J, Laitinen J, Karjalainen A, Hahtola P, Jaakkola JJK. Indoor dampness and molds and development of adult-onset asthma: a population-based incident case-control study. Environmental Health Perspectives 2002; 110(5): 543-547.

Here is a paper from 2002 included to add support to the thesis that by 2002 there was excellent data demonstrating (before the ACOEM report was presented) that indoor dampness and molds were associated with respiratory illness acquisition. This paper shows the fraction of asthma attributable to workplace mold exposure to be 35% among 444,000 people exposed in a hospital district in Finland. The EPA finding of 21% is much lower.

65. Gent JF, Ren P, Belanger K, Triche E, Bracken MB, Holford TR, Leaderer BP. Levels of household mold associated with respiratory symptoms in the first year of life in a cohort at risk for asthma. Environmental Health Perspectives 2002; 110(12); A781-A792.

This paper is referenced in the CDC section showing that by 2002 it was well established that exposure to mold created human health effects at a very high relative risk in patients exposed to water-damaged buildings compared to dry buildings.

66. Apostolakos MJ, Rossmoore H, Beckett WS. Hypersensitivity pneumonitis from ordinary residential exposures. Environmental Health Perspectives 2001; 109(9): 979-981.

This Grand Rounds in Environmental Medicine from 2001 shows that hypersensitivity pneumonitis is associated with exposure to a water-damaged home environment. This paper antedates the ACOEM report by over a year; the prominence of a Grand Rounds in Environmental Medicine can not be underestimated.

67. Trout D, Bernstein J, Martinez K, Biagini R, Wallingford K. Bioaerosol lung damage in a worker with repeated exposure to fungi in a water-damaged building. Environmental Health Perspectives 2001; 109(6): 641-644.

Here is another Grand Rounds in Environmental Medicine from NIEHS calling for further research into concern over health effects related to potential exposure to building occupants to bioaerosols. This case study presents such illness acquisition. The author concluded a systematic clinical approach for evaluating persons with suspected building-related respiratory illnesses is warranted, particularly in the absence of clinical tools specific for evaluation of mycotoxin-related illness. NOTE these specific clinical tools are now readily available to all practicing clinicians.

68. Menetrez MY. Emission exposure model for the transport of toxic mold. Indoor and Built Environment 2004; 13(1); 75-82.

This paper reviews the potential for creation of a bioaerosol of spores and fragment of spores and fungi, both viable and non-viable, from contaminated gypsum wall board. The findings of presence of *Stachybotrys* in emissions with low air velocity flow conditions were found to be directly proportional to the air flow and indirectly proportional to relative humidity. These emission findings corroborate previous observations involving *Penicillium/Aspergillus*. NOTE there are multiple papers supporting the relationship of exposure to spores and fragments of spores from visible mold even in the absence of finding those molds present in a five-minute air test.

69. Craner J. Building-related illness in occupants of mold-contaminated houses: a case series. Eastern New York Occupational & Environmental Health Center 1999; 146-157.

This 1999 paper is an overview of the possible illness acquisition of patients exposed to water-damaged buildings. Though there are only five patients presented, each of these cases illustrated important clinical features and issues in a clinical approach to diagnoses of disorders related to indoor environmental contaminations in the home environment.

70. Engelhart S, Loock A, Skutlarek D, Sagunski H, Lommel A, Farber H, Exner M. Occurrence of toxigenic *Aspergillus versicolor* isolates and sterigmatocystin in carpet dust from damp indoor environments. Applied and Environmental Microbiology 2002; 68(8): 3886-3890.

This is a paper published in August 2002, again well before the ACOEM statement was released, that confirms finding of mycotoxins in carpet tests in areas of homes with water damage. The authors conclude that the *Aspergillus versicolor* isolated from carpet dust was able to produce sterigmatocystin and that this toxin itself was readily isolated from carpet dust. 98% of such isolates of Aspergillus versicolor were toxigenic.

71. Murtoniemi T, Nevalainen A, Hirvonen MR. Effect of plasterboard composition on Stachybotrys chartarum growth and biological activity of spores. Applied and Environmental Microbiology 2003; 69(7): 3751-3757.

This paper from Finland in 2003 looks at the effects of particle board composition on growth and sporulation of *Stachybotrys*. While there are differences in growth on different plaster boards, the biomarker ergosterol correlates with triggering of inflammatory cytokine production in macrophages following isolation. Even use of biocides was inadequate to prevent the cytotoxic potential of spores.

72. Shelton BG, Kirkland KH, Flanders WD, Morris GK. Profiles of airborne fungi in buildings and outdoor environments in the United States. Applied and Environmental Microbiology 2002; 68(4): 1743-1753.

This 2002 paper looked at 12,026 air samples of which 9,619 were indoors from 1,717 buildings located across the United States. These samples were collected during indoor air quality investigations performed from 1996 to 1998. There are geographic differences in fungi found outdoors. There are significant differences, independent of geography, between fungi found indoors in water-damaged buildings compared to non-water damaged buildings and from the water-damaged buildings to outdoor air.

73. Sebastian A, Larson L. Characterization of the microbial community in indoor environments: a chemical-analytical approach. Applied and Environmental Microbiology 2003; 69(6): 3103-3109.

This paper is a follow-up on the use of ergosterol as a marker for fungal biomass. Integrated procedures using gas chromatography and ion trap mass spectrometry were able to show differences in house dust for a variety of compounds. This method could be used to characterize microbial communities in environmental samples. NOTE: the

technology available for exposure assessment was published in the early 1990s as well as the early years of 2000. Researchers are still arguing on how to do exposure assessments.

74. Burge HA. Health effects of biological contaminants. Indoor Air and Human Health Chapter 10 CRC Press 1996; 171-178.

This paper was presented in 1996 at the Oak Ridge National Laboratory Symposium. Burge reviews the known sources of potential for human illness including glucans, cellular fragments and endotoxins, as well as mycotoxins; the lack of evidence correlating presence of those compounds with human illness is discussed. She develops an aberrant hypothetical risk assessment model that ignores individual susceptibility, individual genetics, prior illness of the individual, presence of activation of innate immunity, interaction between compounds found, time of exposure, repeated times of exposure, interactions other than fungal spores with fungi and activation of inflammatory cascades that are non-linear. Despite those absences she comes up with a hypothesis leading to a postulated time it takes to accumulate 1 ng of toxin. This paper serves in some ways as the genesis for the flawed concepts of human illness seen in physician statements expressed in the early 2000s, including that of the CDC.

75. Martinez KF, Rao CY, Burton NC. Exposure assessment and analysis for biological agents. Grana 2004; 43: 193-208.

This review paper from NIOSH focuses on exposure assessments from biological agents. This is a wide-ranging paper that looks at microbes in water-damaged buildings together with microbes that could be used as biowarfare agents. The separation of sampling techniques together with analytical techniques includes discussion of ergosterol, a robust indicator of total fungal biomass, beta glucans, mycotoxins and endotoxins. They call for enhanced use of PCR and DNR microarray.

76. Pessi AM, Suonketo J, Pentti M, Kurkilahti M, Peltola K, Rantio-Lehtimaki A. Microbial growth inside insulated external walls as an indoor air biocontamination source. Applied and Environmental Microbiology 2002; 68(2): 963-967.

This paper looks at communication of air from a covered wall cavity, complete with insulation, to indoor air. There is communication adequate to cause increased presence of actinomycetes in the indoor air coming solely from the insulation layer. The humidity in the wall cavity is a distributing factor that is associated with measurable airborne concentrations of actinomycetes.

77. Krauter P, Biermann A. Reaerosolization of fluidized spores in ventilation systems. Applied and Environmental Microbiology 2007; 73(7): 2165-2172.

This 2007 paper looks at re-aerosolation of spores from HVAC. Re-suspension rates from both steel and plastic duct material are between 10 to the minus 3 and 10 to the minus five per second. There was no difference between metal and plastic duct surfaces. They confirmed that spores that are deposited onto the duct remain a persistent source of contamination for a period of several hours.

78. Thrasher JD, Kilburn K, Immers N. Indoor environment resulting from water intrusion. Perspectives 2006; 4-53.

This overview of the microbes, particulates, mycotoxins, VOCs, hemolysins, beta-glucans and endotoxins that are found in indoor environments are all associated with human illness. The authors conclude "to isolate a single fraction of this environment, in an attempt to indicate from this fraction that adverse health effects upon occupants could not occur is unscientific and irresponsible.

79. Thrasher JD, Crawley S. The biocontaminants and complexity of damp indoor spaces: More than meets the eyes. Toxicol Indust Health 2009; 25:583-625.

This paper reviews the biocontaminants present in WDB resulting from fungi and bacteria and their by-products (particulates, mycotoxins, endotoxins, glucans, extracellular proteins, etc.) They discuss the potential role of *Actinomycetes* and their contribution to illness in the indoors as well as the literature on aspergillosis in immune-competent individuals.

80. Flannigan B, McCabe EM, McGarry F. Allergenic and toxigenic micro-organisms in houses. Journal of Applied Bacteriology Symposium Supplement 1991; 70: 61S-73S.

This 1991 paper breaks out health effects from elements found in indoor air from outdoor air showing increased risk of illness from fungal fragments, fungal volatiles and bacteria in indoor air.

81. Verhoeff AP, van Strien RT, van Wijnen JH, Brunekreef B. Damp housing and childhood respiratory symptoms: the role of sensitization to dust mites and molds. American Journal of Epidemiology 1995; 141(2): 103-109.

This 1995 paper from the Netherlands looks at 259 children with respiratory symptoms and 257 controls. Illness was associated in 94 children with an elevated IgE; 165 children with illness did not have an elevated IgE. The association of health effects following exposure to moldy environments was established in the Netherlands in 1994.

82. Malloy CD, Marr JS. Mycotoxins and public health: a review. J Public Health Management Practice 1997; 3(3): 61-69.

This 1997 paper reviews studies of mycotoxins in public health and calls for public health professionals to use recent developments in the field of mycotoxicology to explore associations between these fungal metabolites and acute and chronic disease in humans.

83. Smoragiewicz W, Cossette B, Boutard A, Krzystyniak K. Trichothecene mycotoxins in the dust of ventilation systems in office buildings. Int Arch Occup Environ Health 1993; 65: 113-117.

This 1993 paper analyzes trichothecene mycotoxins and dust samples from ventilation systems in office buildings. Duct samples were found to have at least four separate trichothecenes, confirmed by high performance liquid chromatography analysis. These

were found with a detectable limit of .4 ng/ml of dust consistent with prior isolation studies showing ease of detection of mycotoxins in buildings with water intrusion.

84. Johanning E, Biagini R, Hull D, Morey P, Jarvis B, Landsbergis P. Health and immunology study following exposure to toxigenic fungi (*Stachybotrys chartarum*) in a water-damaged office environment. Int Arch Occup Environ Health 1996; 68: 207-218.

This 1996 paper looks at exposure to water-damaged buildings and a variety of health effects. It shows the importance of toxigenic organisms and human health associated with inflammatory biomarkers.

85. Johanning E, Landsbergis P, Gareis M, Yang CS, Olmsted E. Clinical experience and results of a sentinel health investigation related to indoor fungal exposure. Environmental Health Perspectives 1999; 107(3): 489-494.

Here is a 1999 paper that follows up Johanning's work from 1996. He summarizes health effects found in 22 children and 125 adults who consulted his clinic in New York together with a table of health effects ranging from respiratory to central nervous system and fatigue. Even at this date in 1999, studies of health outcomes related to bioaerosol exposure appeared more complicated than models of chemical exposure studies because several biological components, beta-glucans, MVOCs, endotoxins, halogens and mycotoxins may have independent or combined effects. This is the first paper to record the observation of a highly increased sensitivity to recurrent fungal exposure noted in some individuals.

86. Rautiala S, Reponen T, Hyvarinen A, Nevalainen A, Vehvilainen A, Kalliokoski P. Exposure to airborne microbes during the repair of moldy buildings. American Industrial Hygiene Asso Journal 1996; 57: 279-284.

This 1996 paper reviews the health effects following exposure to areas of moldy buildings undergoing repair. The concentration of actinomycetes remarkably increased during demolition as well as small particles that carry toxins.

87. Tuomi T, Reijula K, Johnsson T, Hemminki K, Hintikka EL, Lindroos O, Kalso S, Koukila-Kahkola P, Mussalo-Rauhamaa H, Haajtela T. Mycotoxins in crude building materials from water-damaged buildings. Applied and Environmental Microbiology 2000; 66(5): 1899-1904.

This 2000 study from Finland demonstrated the ability to isolate 17 different mycotoxins from 79 bulk samples of interior walls from buildings in Finland with moisture problems and mold growth. Identification and numeration of fungal species present in both materials is possible.

88. Redlich CA, Sparer J, Cullen MR. Sick-building syndrome. Lancet 1997; 349: 1013-1015.

In 1997 Redlich reports a clinical presentation of illness from a moldy building in theUnited Kingdom; she references neurogenic inflammatory responses to low level chemical exposures together with air contaminates.

What is the Relevant Scientific Community?

We often will hear that an unspecified medical community does or does not agree with the idea that exposure to the complex mixture of biocontaminants referenced herein can make people sick. The recent GAO and WHO reports have added significant weight to the arguments of those who treat the immunological problems identified in the human illness acquired following exposure to the interior environment of WDB. Still, for those opposed to the idea of human illness, some of whom have academic appointments or prior government employment, the observer might be persuaded that those opposed to the illness have some greater knowledge of the general medical community than those in favor, as we are told that those in favor of the idea don't have much following in the general medical community. There is no basis in fact for such assumptions.

In litigation, both Frye and Daubert statutes, together with the multiple variations on admissibility used by states, terms like general medical acceptance and use in the medical community are included in the process used by a judge to determine if a given idea is worthy enough to be heard by a jury.

The critical element in determining "the community" is not a sum of all physicians and researchers, as implied by Frye but what are the criteria for determining if the medical community has relevance to the issue of knowledge that will help inform a jury or an agency. Simple possession of an advanced degree, such an MD or PhD does not imbue the possessor with enhanced insight into the issue of human health effects acquired following exposure to the interior environment of WDB. Experience in the issue is paramount, with experience in diagnosis and treatment, maintaining a data base on an ongoing basis certainly qualifies for inclusion in the community. Publication on *human* research and *animal* research also qualifies. Appointment to a committee of a government agency charged with review of the human illness acquired following exposure to WDB also merits inclusion, though the weight of such membership is devalued if there is no evidence of treatment experience or animal research. The role of persons included in a consensus panel, including this one, is fraught with potential bias if there is no evidence of treatment experience or publication on human and animal research. Such consensus statements must be evaluated for their (i) thoroughness of inclusion (ii) rigor of assessment of the science; (iii) scientific accuracy of statements made; (iv) inclusion of pertinent and current scientific literature in this rapidly moving field and (v) absence of concealed conflict of interest. If a consensus panel does not meet each of these five criteria, then the opinion of the consensus must be devalued to the point of elimination.

As an example, it is not uncommon for consultants for defense interests in WDB litigation to have no experience in diagnosis or treatment of the CIRS-WDB. These individuals may have testified in courts of law, providing opinion in such litigation, but that testimony and those opinions alone do not qualify them to be considered as members of the scientific community looking at CIRS-WDB.

Further, prior inclusion in a consensus panel that brought forth flawed information that failed the tests of reliability, thoroughness, rigor, transparency, absence of bias as manifested by failure to disclose conflicts of interest and scientific accuracy weighs against consideration of opinions from those individuals in the future in the absence of papers published in the interim that are peer reviewed studies showing completion of animal or human research studies. Should those members of a prior consensus panel that provided flawed recommendation recant their prior opinions or publish peer reviewed studies supporting their opinions, then they would be eligible to rejoin the reasonable scientific community of CIRS-WDB provided they meet the criteria.

Both the GAO and the WHO meet all five criteria as does this consensus statement. Consensus statements not meeting these criteria include the ACOEM statement of 2002; the AAAAI paper written by Bush et al in 2006; and the ACMT statement of 2007. The biased methods of the ACOEM process have been exposed by two major publications (Craner; Wall Street Journal, (WSJ)). The aberrant logic of the ACOEM was discussed by the IOM (Chapter 4) and by Shoemaker and House (Neurotoxicology and Teratology 2006; see ACOEM and AAAAI critique). Further, the specific language of the ACOEM contains false assumptions as will be specified below. The AAAAI was clearly not thorough; not rigorous; not scientifically accurate (as noted by the GAO the Bush paper eliminated all innate immune abnormalities from consideration of causation of illness; those abnormalities are the very basis for the illness). The ACMT consensus, written by two employees of Veritox, is a clone of ACOEM and AAAAI. The ACMT presented no new knowledge and no consideration of what literature was omitted. As one might suspect, these three opinions are written by persons who are highly paid witnesses for defense in mold litigation. Such a finding of failure to disclose conflicts of interests was decried in a Wall Street Journal expose` written by David Armstrong January 9th, 2007.

Also as one might expect, the three "defense-authored" consensus statements cite no human research and present no animal research to support their opinions. One is reasonably able to wonder, if there is no human or animal data to support the "null" hypothesis, of no illness causation, then what support for "no illness causation" exists?

A survey of published human health studies may help define what the relevant medical community actually is. We present here a series of studies supporting evidence of human health effects in a section called "Association studies" and another section called "Nay-Sayers" in which do not support the existence of health effects associated with exposure to the interior environment of WDB. The weight of the studies confirming presence of illness compared to those that do not is markedly different.

Please note that the Association studies cover 14 countries and over 45,000 patients. The Nay-Sayers present no studies of human health effects.

1. Bernstein, Epidemiology-Literature review, US

Compare Bernstein's comments here in 2007 to those referenced by Sudakin from 1998 in Nay-Sayers. Bernstein now acknowledges that moisture and microbes in buildings can

affect human health by a variety of biological mechanisms. Toxins contribute to the illness. Small particles, called fragments; carry the bulk of inflammagens.

2. Ammann, Epidemiology-Opinion, US

This broad overview of indoor contaminants contributing to health is written by an author of the IOM report.

3. Park, Epidemiology-Case control, Patients-152 cases, US

This NIOSH paper (affiliated with the CDC) concludes that mold levels in dust are associated with new onset asthma in damp indoor environments. Fungi and ergosterol are measures of fungal bio-mass that have promise as markers of risk for building related respiratory diseases.

4. Myer, Epidemiology-Double-blind placebo controlled study, Patients-8, Denmark

Mold exposure is associated with changes in symptoms, though the effect in this small study is too small to rule out an effect of mold itself.

5. Santilli, Epidemiology-Observational, Patients-85, US

Patients with multiple health symptoms were correlated with elevated “accounts” of indoor mold. Symptoms persisted more than two years.

6. Savilahti, Epidemiology-Cross sectional, Finland

Exposure to water-damage building was associated with increased symptoms in children ages 7-12 from two elementary schools. Health records were examined and questionnaires analyzed. After renovation, no difference between cases and controls persisted.

7. Kerscmar, Epidemiology-Prospective randomized control clinical trial, Patients-62 children followed for one year, US

Remediation improved symptoms seen in cases. Symptoms not seen in control patients; no changes noted.

8. Park, Epidemiology-Observational, Patients-college building, US

Exposure response relationships were shown between mold and dampness in buildings and respiratory symptoms.

9. Shenassa, Epidemiology-Logistic regression for cross sectional study, Patients-5882, European countries

The diagnosis of clinical depression was associated statistically with exposure to damp and moldy environments in 8 European countries.

10. Park**,** Epidemiology-Cross sectional**,** Patients-888**,** European countries

Exposure to water-damaged environments significantly increased odds for lower and upper respiratory symptoms. Non-linear relationships were observed for these symptoms when combined with presence of endotoxin in floor dust (NIOSH study).

11. Hope, Epidemiology-Opinion, European countries

This review describes multiple studies that evaluate the association of indoor dampness and mold growth with upper respiratory tract symptoms. The preponderance of epidemiologic studies supports the link between exposure to WDB and development of symptoms.

12. Bornehag, Epidemiology-Literature search, Patients-Academic paper, Sweden

Dampness in buildings increases the risk for health effects in people.

13. Hodgson, Epidemiology-Nested case controlled study, Patients-14, US

Three to 15-fold increases in symptoms in three case buildings compared to two controlled buildings was demonstrated. Neuropsychological measures were increased in workers with exposure with increased levels of symptoms. This study represents "a likely human response to inhaled fungal toxins in indoor environments."

14. Author-Menzies, Epidemiology-Prevalence, Patients-1102, Canada

17% of patients exposed to damp environments had increased health symptoms.

15. Author-Meyer, Epidemiology-Stratified cross sectional study, Patients-1024, Denmark

This association between mold and floor dust with health symptoms showed a strong association in adolescent schoolboys but not among schoolgirls

16. Author-Bornehag, Epidemiology-Multiple; case control and prevalence with follow up, Patients-14077, Denmark

In this study, in which careful attention was paid to the potential for bias, and all confounding health problems ruled out, the study found that symptoms were increased in damp environments.

17. Author-Dales, Epidemiology-Observational, Patients-13799 adults, Canada

A relative risk of 1.62 was seen that associated symptoms and dampness even after controlling for multiple social demographic variables including smoking, type of heat and wood stoves.

18. Wan, Epidemiology-Questionnaire, Patients-1237, China

Dampness in buildings had a dose response effect on eye irritation, cough and fatigue.

19. Author-Cox-Ganser, Epidemiology-Questionnaire, Patients-888, US

This 2005 NIOSH paper finds occupancy of water-damaged buildings was associated with onset and exacerbation of respiratory conditions confirmed by objective medical tests.

20. Cox-Ganser, Epidemiology-Questionnaire, Patients-888, US

Subjects living in damp housing had significant highly prevalence of health symptoms. This association remains significant after adjusting for possible confounders.

21. Jaakkola, Epidemiology-Case control, Patients-531 cases, 932 controls, Sweden

The fraction of asthma attributable to a work place mold exposure was noted to be 35%. Compare this to the EPA number of 21% in the 2007 Fisk study.

22. Gent, Epidemiology-Prospective clinical trial, Patients-880, US

Infants in homes with *Penicillium* demonstrated by airborne samples are at significant risk for wheeze and persistent cough.

23. Apostolakos, Epidemiology-Single case, Patients-1, US

Hypersensitivity pneumonitis can be acquired from residential exposure.

24. Craner, Type-Association, Epidemiology-Case study, Patients-5, US

Five patients developed multiple health symptoms including neurologic symptoms, fatigue and respiratory problems following exposure to water-damaged buildings.

25. Thrasher, Epidemiology-Literature review, Patients-N/A, US

To isolate a single fraction of an indoor environment that contains microbes, particulates, mycotoxins, hemolysins, beta-glucans and endotoxins is unscientific and irresponsible. Human illness caused by fungi and bacteria can result in multiple symptoms including neurotoxicity, respiratory problems, hypersensitivity and immune abnormalities.

26. Johanning, Epidemiology, US

Multiple health effects, including neurologic events and immune abnormalities, follow exposure to toxigenic fungi found in a water-damaged office environment. Multiple health effects were seen in association with exposure to toxic or allergic fungi. Primary diagnoses included respiratory, skin, mucus membranes and central nervous system.

27. Johanning, Epidemiology-Opinion, US

This paper discusses illnesses acquired following exposure to fungal spores. Diseases that are usually associated with inhalation of fungal spores include toxic pneumonitis, hypersensitivity pneumonitis, tremors and chronic fatigue syndrome.

28. D. Mudarri & WJ Fisk and Lawrence Berkeley, National Laboratory, *Public health and economic impact of dampness and mold, Indoor Air 2007*; 17:226-15

These authors conclude: "Effective moisture control in buildings supports public health. There is a general consensus in the scientific community that exposure to dampness and mold substantially increases the risk of a variety of health effects, most notably those associated with the respiratory system. The increased risk to exposed individuals combine with the relatively high prevalence of dampness and mold in buildings means that large numbers of individuals are adversely impacted."

Commentary on papers written by Nay-Sayers

29. Texas Medical Association, 2002, Epidemiology-Opinion, US

Koch's postulates form the basis for proof of infectious and toxic reactions. Large randomized trials are the best standard to make decisions regarding health effects of exposure to water-damaged buildings. Peer-reviewed medical literature should clearly show that mold or mold by-products produce clinical symptoms displayed by a patient.

30. Lees-Haley, Epidemiology-Opinion, US

This writer has appeared with R. Gots. His "Fake Bad Scale," used to impugn the reality of mold illness is widely discredited.

31. Fink, Commentary (on Bobbitt) below, Epidemiology-Opinion, US

Bobbitt describes 135 patients evaluated retrospectively. Less than 50% of patients had site evaluations. No association between allergy and illness was noted. A nine-point scale was used to rate mold exposure; this scale has never been validated or standardized. No control population is presented.

32. Marmot; Whitehall "study", Epidemiology-self-completed questionnaire, UK

This study published in 2006 does not provide any information on illness associated with exposure to WDB. Enrollees from 1991-1993 completed questionnaires regarding health effects by asking about 10 health symptoms, though no correlation with presence of water intrusion was noted. These symptoms did not include aching, pain or cognitive symptoms, among the three most commonly observed problems seen in patients with illness acquired from WDB. Fatigue was seen in only 29% of patients, far lower than 80 + percentage usually seen in cohorts of affected patients. In the absence of any "modern" assessment, this study, published 15 years after the data were collected, has no relevance to any marker of illness or building health. There is no control group or any comparison based on reported microbiologic markers. This study has no relevance to illness acquired from exposure to WDB, as no measures of water damage, no reporting of visible mold or musty smells are reported. The very fact that Nay-Sayers would cite this study as supporting "no illness" creates concern, as this paper has nothing to do with WDB. The paper itself states that, "These findings should not be interpreted as justification for

assuming that the quality of the physical environment is unimportant. Indeed, we cannot leap from a recording of C02, lighting, noise, temperature and air movement and absence of severe health problems in the presence of water damage. This study does not have a role in the WDB literature.

33. Bardana, Epidemiology-chart review, Patients-50, US

Bardana published another paper, similar to this one in 12/05 with his grad student Khalili. He looked at patients for whom he was doing "IME" in legal cases, coming to the conclusions that none of the patients had any verifiable illness. His methods were to look at selected elements of case histories. He claims to have done a comprehensive medical exam; yet no diagnostic studies were ever done. Exclusion criteria eliminated 32 cases of his original 82, for a 39% exclusion rate, indicating selection bias. Studies reviewed included measures of acquired immune response, including IgE, fungal allergy blood tests, pulmonary function tests and methacholine challenges. In no patients did Bardana look for any of the markers of systemic inflammation that verify patients with illness acquired from exposure to WDB. Yet, Bardana concludes that these patients did not have a recognizable illness. He found abundant evidence of allergy. His building surveys were skewed, as none of the buildings were tested for mycotoxins, ergosterol, particulates, glucans, proteases or endotoxins. His methods do not permit any comment on the role of systemic inflammation on illness.

Note: Dr. Bardana's study is further weakened by inclusion of one of Dr. Shoemaker's patients who saw Dr. Shoemaker after seeing Dr. Bardana. Her "comprehensive exam" from Dr. Bardana's research colleague consisted of a 10-minute interview. Shoemaker treated the patient with excellent initial results. She completed a repetitive exposure protocol that confirmed that she developed marked abnormalities in inflammatory responses within hours of re-exposure, off medication.

34. Author-Bobbitt, Epidemiology-retrospective chart review, no control group, Patients-135, US

Bobbitt states that his 9-point exposure scale has merit. No other medical paper uses that scale. His testing looked for IgE, skin testing and pulmonary function testing. He did no testing to look at systemic inflammatory illness. He found that non-allergic patients were much more likely to have lower respiratory symptoms and systemic symptoms. Retrospective studies must discuss the epidemiologic approach that permits a reader to be assured that bias was not a factor in the analysis. There is no such evidence here.

35. Robbins 2000 (AKA Veritox I), Epidemiology-Opinion, US

This is the Gots/Kelman study, published in 2000, the apparent basis for the opinions for ACOEM, AAAAI and ACMT. The paper reports no accepted epidemiologic standards for its methods. The assumption is that the main route of exposure of humans to mycotoxins is ingestion. This statement has no cited references for humans. Looking at previously published studies on one-time exposure to animals to trichothecenes, the authors find significant organ damage in the animals. They readily admit that inhalational studies "do not represent exposure to mycotoxins at chronic, low exposure

levels from molds in indoor settings." They then form the conclusion that existence of a threshold for the level of mycotoxins follows because of "decreasing toxicity with longer exposure for a given total dose." This conclusion bears no relationship to any logical pathway known. There is no basis to assume that ongoing chronic exposures are identical in people to what was seen in a fixed total exposure in animals in which no other exposure except to mycotoxins was performed. The illness stems from exposure to a complex mixture of inflammagens and toxigenic organisms found in WDB. The study of Nikulin is cited. Unknown amounts of toxins were injected intranasally (not intra-tracheal administration) into mice one time. All mice had marked lung inflammation, with the more toxic spores injuring the mice more. In a twice-weekly inoculation experiment that lasted for three weeks, Nikulin again showed significant lung damage related to toxicity of the spores. The authors agree, "Intranasal inoculation is unlikely to model the exposure of humans in even very moldy environments." This is true, though not for the reasons the authors want to argue. The authors discuss a paper from Johanning, trying to find no dose-response relationship by looking at populations of white blood cells. They do not look at the bulk of the evidence of inflammation in any other study. The authors conclude that "the issue of mold exposure is important from a health standpoint and can potentially affect anyone in the indoor environment."

36. Kelman, 2004 (AKA Veritox II), Epidemiology-Opinion, US

Kelman is a co-author of the study above. This is the study labeled as junk science (and therefore not acceptable) by California (see case citation and quotes) and rejected as baseless in a Frye-Reed hearing. However, it is cited by all the AAAAI and ACMT authors. Here the word, "implausible" is used to describe illness, when actually the word implausible applies only to the methods and unsupported step from one-time studies in animals to human illness. Modeling of exposure without data is merely a guess. The authors agree that "the relevance to human inhalation-response is uncertain for non-physiologic dosing regimens and for ex vivo measures of uncertain relevance to human health." Nonetheless, they ignore the cascade of inflammatory responses that come from differential gene activation, receptor activation and interaction of innate immune elements with others. The illness acquired from exposure to WDB as identified by the authors of this position statement bears no resemblance to what these authors bring forward.

37. ACOEM, Epidemiology-Opinion, US

Written by Kelman and his business partner, Hardin, with defense consultant Dr. Saxon, this paper is extensively criticized by many, including Ammann, Shoemaker and House (NTT 2006) and the IOM. None of the 83 references supports the claim that human health has not been adversely affected by inhaled mycotoxins. Of interest is the language on page 475 that is quoted from the Texas Medical Association (TMA) without attribution. ACOEM and TMA, as well as the first CDC statement on mold, were all released essentially simultaneously in 2002. This paper demands that there is absorption of a toxic dose over a sufficiently short period of time. There is no reference for this statement on page 474. The authors cite the Creasia studies that show acute toxicity from non-physiologic exposure of animals and the Nikulin study discussed above. Neither of

these studies supports illness-finding in chronic low dose exposure of humans to conditions in WDB. Further, the authors erroneously claim (again, without reference) on page 474 that cumulative dose delivered over hours, days or weeks is expected to be *less toxic* than bolus. This paper remains conjecture at best, made without academic basis or any experimental data to support the conclusion invariably used in litigation that exposure to WDB, complete with all the factors that create inflammation beyond mycotoxins, couldn't make people sick.

38. Sudakin, pre Veritox, Epidemiology-none, US

Presence of elevated levels of molds was associated with illness symptoms. Such symptoms were reduced after the building was cleaned up.

39. Sudakin, pre Veritox, Epidemiology-none, US

This review simply suggests that good scientific studies need to be done to show illness from WDB. Such studies are now done.

40. Author-Ghannoum, Epidemiology-none, US

Dr. Ghannoum testifies often for defense in mold litigation. Nonetheless, he states that dampness and mold-associated illnesses, including those caused by *Stachybotrys*, have a great impact on health and the economy.

41. Ghannoum, Epidemiology-none, US

Illnesses caused by indoor mold included pulmonary, immunologic, neurologic and oncologic disorders. Measuring mycotoxins is difficult. He calls for physical exams and diagnostic testing (pg 147); our studies and those of collaborators all do such procedures. He calls for studies with objective markers of illness, proper epidemiologic techniques and careful examination for bacteria, endotoxins, man-made chemicals and nutritional factors.

42. Terr, Epidemiology-literature review, US

Terr acknowledges that biologic effects of fungal toxins include cytotoxicity, metabolic effects, hemolysis, plasmin effects, pulmonary effects and immunologic effects. He concludes however, that low level exposure to *Stachybotrys* could not cause illness. He cites no studies to support his conclusion. In addition he did not recognize or discuss the biological complexity that exists in WDB.

43. Kolstad, Epidemiology-opinion, Denmark

He notes multiple health effects, especially respiratory. He focused on IgE, a factor important in acquired immune responses, ignoring innate immune responses. He calls for studies that employ inflammatory markers to avoid reporting bias. Our studies use these approaches.

44. Fung, Epidemiology-literature review, US

Fung was a defense consultant in mold litigation. He calls for additional studies to establish exposure response relationships. These have been accomplished.

45. Paper-Mahmoudi, Epidemiology-none, US

Part of the Ghannoum connection, this paper from 2000 calls for prospective studies to assess the relationship of exposure to illness.

46. ACMT, Epidemiology-none, US

This consensus statement, authored by an employee of Veritox, Dr. Sudakin, uses three references to support its conclusion that exposure to mold doesn't cause inflammatory illness. One is ACOEM; another is Kelman 2004 and the last is the excellent study showing direct neurotoxicity from exposure of animals to fungal toxins presented by Dr. Pestka's paper. Because Pestka's study actually refutes Dr. Sudakin's thesis, it remains unclear as to why the paper is included by ACMT as a supporting reference, as it clearly shows why exposure to WDB creates a tremendous health burden.

47. Bush, Epidemiology-none, US

This oft-quoted defense instrument was widely criticized by a series of 10 letters published in JACI 09/06. Written by Saxon and Terr, among others, it was reported by the Wall Street Journal 01/09/07 that egregious conflicts of interest were withheld from view. The consensus of criticisms resulted in the JACI revamping its conflict of interest policy; provide an editorial comment that the paper wasn't the last word; and acknowledged that one of the co-authors of the draft, Dr. Portnoy, withdrew his name from the paper in protest. This paper provides no human health data, instead relying on ACOEM, Kelman and Robbins. The paper was defended by its authors by saying that less than 1% of the AAAAI membership criticized the paper. The approach of JACI to publish 10 letters sharply critical of Bush is unprecedented.

Why No One Should Accept the ACOEM and AAAAI Consensus Statements

This document acknowledges the existence of differing opinions (called "Nay-Sayers") from those expressed in this position statement regarding the subject of human illness acquired following exposure to the interior environment of WDB. Essentially the ideas of the Nay-Sayers can be reduced to just a few concepts, each espoused by two consensus statements published by (i) the American College of Occupational and Environmental Medicine (ACOEM, 10/2002) and (ii) the American Academy of Allergy, Asthma and Immunology (AAAAI, 2/2006).

The differences between opinion of treating physicians and Nay-Sayers are many. These include but are not limited to:

(1) Patients can be identified with objective laboratory parameters as having a CIRS-WDB that responded to therapies that could help no other illnesses.
(2) Nay-Sayers exclude clinical experience with real patients; they have no research basis to support their comments
(3) Nay-Sayers ignore the epidemiologically accepted concepts of causation and causality, particularly with regard to prospective documentation of acquisition of illness
(4) Nay-Sayers deny multiplicity of components in the WDB that is the actual "dose" of exposure
(5) The Nay-Sayers try to suggest that ingestion is the source of illness
(6) Nay-Sayers ignore the immunological basis of the illness associated with activation of exponentially expanding cascades of host responses
(7) Nay-Sayers deny existence of differential genetic susceptibility to the illness
(8) Treating physicians identify an individual as a case or not, which may occur within a group that is evaluated
(9) The WDB is a unique ecological niche that has not been reproduced in laboratory environments to date and is not replicated by single dose, acute exposure studies

Based on the numerous references annotated, the authors of this statement feel (i) that the ACOEM and AAAAI papers must be held to the same standard of academic integrity as all others; and that (ii) the two papers are academically without merit; and (iii) should not be given weight in litigation.

Of significant concern, these papers have been deemed biased and flawed by two publications (Craner, 2008 and Wall Street Journal, 1/9/07) and rely heavily on the fundamentally non-scientific opinions expressed in the Gots/Kelman, 2000 paper. Major errors in these papers include i) the assertion that mycotoxins alone are the agents responsible for human illness; ii) that the innate immune response is irrelevant; iii) that no human health effects are possible from exposure to WDB; iv) that ingestion, not inhalation, is the primary source of exposure; v) that single dose inhalation studies in rats

are comparable to chronic low dose exposures in humans; vi) that there is a linear dose response to exposure; and vii) that it is possible or even scientifically relevant to analyze a single component of exposure from what is found in WDB.

Presented below is a point by point refutation of the validity of these papers.

Standard of Academic Integrity

These two documents must be held to a standard of academic integrity with (i) absence of bias; (ii) presence of thoroughness; (iii) presence of academic rigor; and (iv) presence of transparency in decision making, especially including peer review and revelation of conflicts of interest. These standards are found to be lacking on each point in each paper.

A comprehensive summary of the events that led to publication of the ACOEM statement, including decisions made by the executive board of ACOEM that solicited the statement, was written by James Craner MD, and recently published in the International Journal of Occupational and Environmental Health. Dr. Craner's expose' of the flawed process and flawed science used by the ACOEM authors has provided the transparency need to discredit the ACOEM opinion. Per Dr. Craner's article, it appears as though a deliberate attempt was made to deceive not only the members of ACOEM, but also jurists and the public at large. As Dr. Craner and also the Wall Street Journal write (1/9/07), there were concealed conflicts of interest of the authors of the ACOEM.

As of March 2009, ACOEM stated that it intended to revise its 2002 statement, but by July, 2010, no such revision is published.

The AAAAI revised their policy of disclosure of conflicts of interests following a series of papers published 9/06 in JACI critical of the absence of conflict of interests statements in the 2/06 AAAAI consensus statement.

Conflicts of interest and academic bias are not the only problems with these papers however. Serious methodological mistakes and leaps of logic to conclusions (not supported by the US GAO and WHO opinions) are found in the studies (animal and others) cited in the paper. The "father of all the Nay-Sayers opinions," the Gots/Kelman, 2000 study, will be discussed in its own section. In the paragraphs that follow we list examples of mistakes with others discussed in the ERROR section below.

As discussed in the published paper from Center for Research on Biotoxin Associated Illnesses in Neurotoxicology and Teratology 8/7/06, anyone who looks at the ACOEM report must also look at the methods it used before even considering reading the conclusions. The methods of a study set the standard for what conclusions can be drawn. The report relied on one study involving a one-time, short-term exposure of Sprague-Dawley rats to washed fungal spores. No recording of toxin amounts was included, nor was any delineation made of the ability of the selected species of "toxigenic" fungi to actually make toxins. Given these lapses, one has to question the utility of the study, considering that it purports to de-link the observed chronic illness to exposure to

concentrations of spores and toxins, even though it lacks basic baseline parameters required to investigate the link. One may make no conclusions about toxicity when the study involves unknown concentrations of spores and toxins; unknown ability of the fungi in question to actually make toxins; and absence of any validated marker for assessment of toxin effect.

The parameters used in the ACOEM paper bear no relationship to the actual findings in patients exposed to water-damaged buildings. The level of detection (200x magnification) used to survey for fungal elements is too insensitive (it must be at least 800x), thereby missing a substantial portion of fungal elements that carry toxins, perhaps by as much as 90%. According to NIOSH/CDC, small elements carry as much as 99.8% of the total burden of toxin and inflammagen found inside WDB.

Both the ACOEM and AAAAI reports extrapolate from observation of acute effects, using those data to then **leap to the conclusion** that there is a threshold for the level of mycotoxins causing injury. They then compound this error in logic by assuming that there is "decreasing toxicity with longer exposure for a given total dose." Which facts underlay this unreferenced statement? The authors **do not cite any studies** to support this statement because there are none. **There is no "decreasing toxicity" with longer exposure**. In fact, as shown by published data, there is recruitment of ***more*** inflammatory pathway components leading to further expansion of the illness to include injury to production mechanisms of regulatory hypothalamic neuropeptides.

This unfounded leap of logic, concealed in the verbiage of the ACOEM statement, bears no relationship to any known biological pathway. Clinicians who actually treat patients with CIRS-WDB agree that the physiology of the illness involves enhanced response (reactivity) with repetitive exposure. This ACOEM statement to the contrary demonstrates their lack of experience possessed by the authors in diagnosis and treatment of CIRS-WDB; indeed, none have either documented their research or even data collection in scientific or public arenas.

The fallacy behind the attempts of the ACOEM and AAAAI papers to deny the reality of CIRS-WDB is well-demonstrated by the report from CRBAI of 850 cases and 132 controls, accepted after peer review for the 9th International Health Buildings conference (9/09). The study of these patients, by far the world's largest study of innate inflammatory responses seen in affected patients, expands the older case definition of biotoxin-associated illness to that of a chronic inflammatory response syndrome. This paper, followed by another, yet even more complete study of 815 patients and 130 controls (CRBAI, International Mycology Conference, 8/1/10) increases the total number of patients (adults and children) reported in current literature by CRBAI to be 2,030 cases and 450 controls within six publications.

The studies cited in the ACOEM and AAAAI paper are not suitable for chronic risk assessment purposes: they are one-time, acute animal exposure studies and do not include adverse effect parameters. The only study the ACOEM and AAAAI opinions cite ***didn't identify toxins*** or the specific strain of *Stachybotrys* used in their experiments. The doses

of toxic exposures were apparently large with no attempt made to identify the maximum or minimum level of dose tolerated. The rats in the study were sickened by their exposure, with bleeding into the lungs noted. The ACOEM and AAAAI opinions have no toxicity data and no exposure data. There is no logical rationale for applying an extrapolation of animal data to human data in this study. Note that we must recognize that the effects of exposures on human infants and children are not the same as in adults. The ACOEM and AAAAI reports never explain the differences between toxins found in indoor water-damaged buildings or whether the toxins were from molds, actinomycetes, mycobacteria or bacteria. Indeed the reader is expected to accept the unproven and unreferenced allusion to "aflatoxin equivalents" as sound science. It is not.

Further, the two statements do not recognize the presence of fine particulates mentioned above that are shed by microbial colonies.

The lack of transparency demonstrated by failure to reveal conflicts of interest severely damages the credibility of the ACOEM paper. The AAAAI paper cites the ACOEM opinion. The questionable methods and unsupported conclusions contained in studies cited by both these consensus statements remove all credibility from both reports.

Validity of Gots/Kelman 2000

The ACOEM statement relies heavily on the scientifically flawed paper written by defense consultants Bruce Kelman and Ronald Gots (see reference to Gots/ Kelman, 2000). The ACOEM and AAAAI opinions make no attempt to re-evaluate the faulty methods and unsupported ideas of the parent paper. Indeed it is important to not forget that the *foundation from which all mold papers were based followed the erroneous path initiated by Gots/Kelman*. This trail of self-perpetuating inaccuracies resulting in irrelevant conclusions was blazed by the authors. ACOEM cites Gots/Kelman; AAAAI (Bush) cites ACOEM; ACMT cites ACOEM and AAAAI. The validity of all these papers is tainted by the respective authors' working relationship with prominent firms that provide support to defense interests in mold litigation.

The Gots/Kelman paper reports use of no methods for its conclusions. The paper uses no accepted epidemiologic standards for its approach. Their **assumption** is that the main route of mycotoxin exposure in humans is ingestion. A quick look at any of the governmental agencies statements regarding mold illness or any of the papers on over 50,000 mold patients definitively demonstrates the importance of inhalation as the mode of acquisition. The statement regarding ingestion in the Gots/Kelman paper **has no cited references for humans**. Looking at previously published studies on one-time inhalation exposure to animals to trichothecenes, significant organ damage in animals is reported. Apparently, observers are supposed to ignore the adverse health effects seen in the test animals from such exposure. Gots/Kelman readily admits that such one-time *inhalational studies* "do not represent exposure to mycotoxins at chronic, low exposure levels from molds in indoor settings."

The AAAAI paper has been extensively criticized by many, including Ammann, Shoemaker and House (NTT 2006) and the IOM. None of its 83 references supports the claim that human health has not been adversely affected by inhaled mycotoxins. The ACOEM report and the first CDC statement on mold were both released in the fall of 2002. The Gots/Kelman paper posits as fact that there must be *absorption of a toxic dose over a sufficiently short period of time*. There is no reference for this statement, as there cannot be! **It simply is not true**. Chronic low-dose exposure occurs over longer periods of time, not a "sufficiently short period of time." AAAAI cite studies (Creasia) that show acute toxicity from non-physiologic exposure of animals. Neither of these studies analyze illness-finding in **chronic low dose exposures** in humans. Further, the authors erroneously claim (again, without reference) that the cumulative dose delivered over hours, days or weeks is expected to be ***less toxic*** than single delivery of a bolus of spores. Upon what basis does this illogical assertion rest? Illness acquired following exposure to the interior environments of WDB is clearly based on low level, chronic exposure to humans. The Gots/Kelman paper discussed high-dose acute exposures of animals to unspecified amounts of unspecified toxins found on mold spores. As such, their findings have no relevance to the demonstration of whether or not long term exposure to WDB can cause illness in humans. We note that in litigation, defense "experts" invariably quote these papers (Gots/Kelman, ACOEM and AAAAI) as "proof" that exposure to WDB couldn't make people sick.

One should wonder how such "junk science," as labeled in a California ruling (Harold v. California Casualty No. 02AS04291, 2006), based on extrapolating data from one study of acute, high-dose exposure to unknown mycotoxins in rats can lead to the drawing of conclusions about the absence of human illness in association with chronic, low-dose exposure to water-damaged buildings. Yet these misleading and deceptive documents are cited repeatedly by a small cadre of non-treating physicians who consider them to be acceptable science. Unfortunately, "junk science" leads to erroneous conclusions regarding CIRS-WDB. By citing the first "junk science" paper at the start of a discussion, then adding the ACOEM opinion, itself based on "junk science," and then adding a third "junk science" paper, litigators thereby try to suggest that a "robust literature" supports the idea that mycotoxins alone would be responsible for any symptoms of illness. This idea is not supported by any academic organization and is not supported by any government agency. Specifically the US GAO and WHO opinions directly disagree. The actual robust literature is that cited throughout this statement.

The AAAAI report ignores the absence of any support in any literature that looks at human research. The GAO report points this deficiency out by noting that Bush included no data on actual research subjects.

Error #1 - Mycotoxins Alone Responsible for Illness

The ACOEM and AAAAI papers suggest that mycotoxins alone would be responsible for any symptoms of illness. This idea is not supported by any academic organization and is not supported by any government agency. Specifically the US GAO and WHO opinions directly disagree.

The AAAAI report ignores the absence of any support in any literature that looks at human research. The GAO report points this deficiency out by noting that Bush included no data on actual research subjects.

Error #2 - Innate Immune Responses are Unimportant

The ACOEM and AAAAI opinions would have us believe that immunologic mechanisms, specifically innate immune responses aren't important in pathogenesis of the illness symptoms. This idea is not supported by any academic organization and is not supported by any government agency. Specifically the US GAO and WHO opinions directly disagree. **Bush, in fact, suggests (pg 329) that observers *ignore the innate immune responses* from building exposure that are the illness!** The GAO statement points out this clear attempt at obfuscation by noting that Bush ignored the immunological features that follow exposure to mycotoxins. The WHO report also weighs in on the immunological aspects of this illness.

Error #3 - No Human Health Effects Are Possible

The ACOEM and AAAAI papers lead us to believe that no human health effects are possible following exposure to the interior environment of WDB. This idea is not supported by any academic organization and is not supported by any government agency. Specifically the US GAO and WHO opinions directly disagree. The lack of validity of the Gots/Kelman, 2000 study on this point has already been discussed. Again, the ACOEM opinion relies on Gots/Kelman and the AAAAI statement cites the ACOEM report.

Note that the ACOEM and AAAAI papers specifically ignore published human and animal health studies that directly contradict the opinions of ACOEM and AAAAI. Note also that they make no attempt to refute the documented physiologic studies published from around the world showing mechanisms in the CIRS-WDB. Finally, note that the ACOEM and AAAAI reports do not cite any studies reporting any physiologic studies; or any prospective studies done in humans or animals that refute the universally acknowledged documentation of illness reacquisition with re-exposure.

Error #4 - Ingestion of Mold is the Mechanism

The ACOEM and AAAAI papers tell us that ingestion is the mechanisms of illness acquisition. This idea is not supported by any academic organization and is not supported by any government agency. Specifically the US GAO and WHO opinions directly disagree.

This idea comes from the previously discussed paper written by defense consultants Gots/ Kelman in which they *assume* that mold illness is acquired following *ingestion* of mold spores and products. There is no basis for this assumption and indeed no one other than

the few collaborating defense consultants suggests that CIRS/WDB comes from ingestion by eating and swallowing mold. All government agencies and researchers in the field agree that inhalation is the primary mechanism of exposure.

The ingestion idea is an artifact from the history of mold contaminated foods, the perception of which is allowed to continue within the artificially created void which excludes relevant evidence.

Error #5 - Single Exposure High-dose Inhalation Rat Studies are Comparable to Chronic Low-dose Human Studies

The ACOEM and AAAAI statements would have readers believe that a single dose, inhalation exposure study of spores instilled into the trachea of rats (which reported severe inflammatory responses in the test subjects, even after washing spores with methanol) is equivalent to the long term, sub-acute exposure of patients to the interior environment of a WDB. Consider that the study cited by the ACOEM and AAAAI papers used spores that were washed with methanol to reduce presence of pathogenic substances including toxins. The data was then extrapolated to support assessment of physiologic findings in humans with chronic low dose exposures. They manufacture the idea of "no effect" by deleting what actually happened to the rats. The "no effect" idea is not supported by any academic organization and is not supported by any government agency. Specifically the US GAO and WHO opinions directly disagree.

An important paper (Neurotoxicology and Teratology 2006; 28: 573-588. SBS and exposure to water damaged buildings: time series study, clinical trial and mechanisms. Shoemaker R, House D.) attacked this study with specific point by point refutation as follows:

1. A no-observable adverse effect level (NOAEL) was not identified in the rodent study. Pulmonary inflammation may occur at dosages below $2.8x10^5$ spores/kg body weight. Another study of fungal-induced pulmonary inflammation estimated the NOAEL to be $<3.0x10^4$ spores *S chartarum*/kg body weight.
2. Airborne fungal spores carry only a fraction of the mycotoxins and other biologically active mixture components to which people are exposed in WDBs. The concentration of small fungal fragments carrying mycotoxins, antigens, and other biologically active components exceed spore concentrations by up to 500-fold. For example, concentrations of air-borne trichothecenes carried primarily on fungal fragments smaller than intact conidia were reported to exceed 1300 pg/m^3 in WDBs.
3. Young adult mice were used in the rodent studies. Younger and older populations, as well as other physically compromised populations, may be more susceptible to exposure-induced inflammation than healthy, young adults.
4. The initial onset of Sick Building Syndrome (SBS) is typically observed in occupants of WDBs following chronic exposure, often many months after

exposure begins. Human health risk assessments for SBS should not be based on effect levels from sub-chronic rodent studies without consideration of uncertainty factors. Uncertainty factors include the potential for cumulative effects, toxin accumulation in tissues and effect threshold shifts to lower levels as protective and repair mechanisms are compromised during chronic exposure. Additional uncertainty factors include interspecies differences in susceptibility and intra-species differences including genetic polymorphisms affecting toxin elimination. Amplification of the pro-inflammatory cytokine response by rising levels of leptin and blockage of the proopiomelanocortin (POMC) response may also be an important factor in the progression of illness during chronic exposure that may not fully develop during acute and sub-chronic exposures.

5. Previous episodes of SBS from exposure to WDB may sensitize patients to subsequent exposures. The hypothesis of sensitization is supported by the observation of relapse within 3 days of re-exposure in the current and previous studies, as opposed to the gradual onset of initial illness reported by the study participants.
6. The potential for additive and synergistic induction of a pro-inflammatory cytokine response by mixture components indicates that human health risk assessments for SBS should be based on studies of exposure to mixtures actually observed in WDBs. Studies should determine the NOAEL for development of an inflammatory response during initial acquisition of SBS following chronic exposure to the mixtures and the concentration and time dependence of acute exposure-induced reacquisition of SBS following cholestyramine (CSM) therapy and subsidence of the pro-inflammatory cytokine response.

In the same rat study the AAAAI and ACOEM statements cited as "definitive," the Rao and co-authors specifically state that *no conclusions* about effects of exposure of people in low dose exposure over time *can be drawn*. In addition, the **authors** of the rat study **themselves** say that extrapolation **cannot be made** from the results of the limited, one-time, high-dose exposure in animals to long-term, low-dose exposure in humans. Indeed, they say the results aren't the same as in chronic low-dose exposure.

Despite these obvious and widely acknowledged limitations that would bar any application to human illness and based on one high-dose study in rats looking at unknown mycotoxins only, against the rat study's author's advice, the ACOEM authors conclude that there is no way an adequate dose of toxins from indoor exposures to the complex biological mixtures of toxigenic fungi, bacteria and actinomycetes can cause human illness in chronic exposure.

This illogical conclusion is not supported by any academic organization and is not supported by all government agencies. Specifically the US GAO and WHO opinions disagree with ACOEM and AAAAI.

Further, the rat study had more shortcomings. For example, the study did not present any data on what toxin exposure actually does to inflammatory cascades; it didn't report any

follow-up on the rats. The methods of the study excluded any reasonable assessment of what material/toxins were delivered to the rat subjects. All the study did was to show that instillation of unknown doses of *Stachybotrys* spores into rat lungs injured the rats severely. The ACOEM report discussed none of the rat study's deficiencies and incorrectly attempted to apply dose-response relationships to elements that could never support such conclusions, especially from chronic, lower intensity dose exposures.

As would be expected, the rat study cited in the ACOEM report has never been replicated. Indeed, the study has been refuted in multiple studies done by Dr. Thomas Rand's group from Nova Scotia which show significant activation (100,000-fold) of inflammatory cytokines by **as few as 30 spores** of toxigenic fungi per gram in mice following intra-tracheal instillation.

Of note, Dr. Rand didn't wash away the toxins from mold spores before instillation and he did measure pertinent inflammatory markers. His data on hyperacute exposure apply directly to what we see in humans: inflammatory responses are measurable in affected patients beginning within hours of exposure to contaminated structures.

Finally, there is no basis to assume that ongoing chronic exposures in people are equivalent to what was seen in a fixed total exposure in animals in which no other exposure except to mycotoxins was performed. **CIRS-WDB stems from exposure to a complex mixture of inflammagens and toxigenic organisms found in WDB.** The study of Nikulin is also quoted. Unknown amounts of toxins were injected intranasally (not intra-tracheal administration, for unknown reasons) into mice one time (only). All mice again had marked lung inflammation. In a twice-weekly inoculation experiment that lasted for three weeks (this is not a chronic, low-dose exposure study design), Nikulin again showed significant lung damage, all the while noting the absence of applicability of the Nikulin study to real world human illness by stating: "intranasal inoculation is unlikely to model the exposure of humans in even very moldy environments."

Curiously, and out of character for the rest of the paper, ACOEM notes, "the issue of mold exposure is important from a health standpoint and can potentially affect anyone in the indoor environment."

Error #6 - Dose Response to Exposure is Linear

The idea that the "the dose makes the poison," permeates toxicology. For a given dose there will be a given and proportionate response. This idea, called a monotonic response, is not applicable to first, activation of pattern receptors following antigen detection; and second, differential gene activation by initiated by such detection. The activation of initial responses leads to profound host innate immune responsiveness that is ***exponentia***l and actually *becomes* the illness.

As opposed to the idea from toxicology that the "poison is in the dose," the concept here is that the poison is in the ***initiation*** of the mega-multiplying response to a given signal.

As Dr. Lewis Thomas has said repeatedly, "the **response of the host** makes the disease." The host response is the illness and not the antigen. Said another way, Dr. Thomas rightfully tells us that "the reaction of sensing is the clinical disease" (NEJM 1972; 287: 553-555) and, "we are in danger from so many defense mechanisms, that we are in more danger from them than from the invaders." And so it is with CIRS-WDB patients.

We are entering an era of understanding of how chronic inflammatory response syndromes impact on the assessment and treatment of chronic fatiguing illness. Dr. Thomas showed us the way to address the importance of host innate immune responses. Results of both animal research and human research studies are consistent with the actual human data revealed (i) in assessment of baseline profiling of cases and then (ii) in responses to therapy. Exceedingly small exposures can set off massive immune responses: this is what treating physicians see on a daily basis in patients with CIRS-WDB. The ACOEM and AAAAI papers reported neither actual human data nor any results of human research; of course their conclusions are without weight.

No single element can ever be identified as causative in CIRS-WDB illness. Specific causation cannot possibly be assigned to any element of the complex mixture found inside the interior environment of WDB that contain multiple contaminants resulting from the presence of multiples species of fungi, actinomycetes, mycobacteria, Gram positive and negative bacteria and their by-products. Thus, the "dose makes the poison" concept cannot be applied to the diverse conditions of WDB as there is **no single dose; instead there is a multiplicity of components within the naturally undifferentiated dose.** Finally, genetic variability, age, and pre-existing health conditions of occupants add to the complexity of CIRS-WDB illness.

It is this observation of repetitively (and consistently) observed differential host responses that underlies published repetitive exposure studies.

The final blow to linear dose response arguments:

Consider that an effect or response (X) is related in a linear fashion to dose. (X) will then be equal to the sum of routes of exposure (A) plus contaminants (B) plus length of time of exposure (C) plus individual genetic susceptibility (D) plus individual prior exposure and change of susceptibility from that exposure (E) plus types of microbial organisms, each potentially acting synergistically with another (F) plus the types of inflammagens causing potentially exponential changes in c-type lectin receptors, especially dectin-1 and dectin-2 receptors. (G) In addition, particular compounds, including mannosylated glycoproteins made by fungi can activate mannose receptors (H) that then alter the signal given to antigen recognition cells to respond to such antigens, further altering the processing of antigen in the intracellular components (endoplasmic reticulum and Golgi body) of such cells. X then is equal to the combined effects of A through H, each of which can cause *amplification*, *not addition*, of the effects of innate immune responses. Moreover, the elements A through H are each themselves variable. The implications of this analysis gets worse for the linear dose-response advocates: there are interactions of A through H, some of which are synergistic and some of which involve differential gene

activation as well as epigenetic phenomena. Proponents of a linear dose response cannot account for how their model can withstand the above discussion. *It is impossible to assume that response or effect X will be linearly related to variables, each simultaneously expressed in A through H.*

Assumptions about dose responses being linear, or even worse, attempt to portray dose responses as active and reliable, as depicted in the ACOEM and AAAAI papers; do not have any validity in scientific fact. **Bush, in fact, suggests (pg 329) that observers *ignore the innate immune responses* from building exposure that are actually the illness!**

Error #7 - Attempts to Assess Specific Causation

No one **can analyze one component of exposure inside WDB**, namely mold spores or mycotoxins, as suggested by non-treating professionals in various groups, and come to any meaningful conclusions from classical monotonic dose-response relationships when the health problems seen repeatedly stem from the exponential, ever-expanding host response to exposure.

Said another way, the complex mixture of exposure and the altered susceptibility of the host resulting from prior exposure to WDB, combined with the complexity of pattern recognition responses of innate immunity results in an extraordinarily amplifying immunologic response by the host to new, even short-term, low level exposures within the WDB. In order to understand the biological cascade of responses to exposure to a WDB, we must assess the potential and actual responses of each **host**. This idea is a fundamental shift in the "Sick Building" paradigm. Prior focus on the individual components of the complex mixture in the building has been superseded by focus on the individual within the building.

Observers must recall that by 2004 we knew that the accumulated mass of fragments of spores was the reservoir in which toxins were found. In 2007, research advances discredited previous erroneous statements about dose-response relationships in CIRS-WDB; these revisions were then incorporated into public policy. Research scientists from the CDC, as mentioned, published a paper in Applied and Environmental Microbiology (March 2007) that confirmed the association of health effects to exposure to WDB. In the acknowledgments to this paper, the authors expressed gratitude to the Inspector General of the Department of Health and Human Services for providing **protection to safeguard the health** of the samplers. One might ask, "Protection from what?"

Moreover, the Ministry of Health of Canada (Canada Gazette 2007-03-31 Part I, volume 141, No. 13) concluded that "results from tests for the presence of fungi in air cannot be used to assess risks to the health of building occupants." Further, the Minister recommended that individuals, "control humidity and diligently repair any water damage in WDBs to prevent mould growth," and that all such WDBs should be subject to the directive to, "clean thoroughly any **visible or concealed mould** growing in WDB

buildings." In a major monograph on indoor air quality published in Volume 115 of Environmental Health Perspectives June 2007, editorial writer Bob Weinhold traced the changes in thinking about mold illness and wrote on page A305: "Regardless of the remaining uncertainties, the overall recommendations of many organizations and agencies worldwide are reaching a common conclusion: **Don't mess with mold. If you can see it or smell it-and especially if health problems are occurring-clean it out, throw it out, or get out."**

CIRS-WDB involves so many inflammagens and toxins that trying to study the unique ecological niche that is a WDB with just one parameter doesn't make sense. What occurs inside a building is not the same as what occurs outside the building. A building is not a compost pit or a pile of wet leaves, for example. Moreover, our understanding of just what is in the affected buildings continues to grow with the advance of research. Just two years ago, no one talked about mycolactone-forming mycobacteria and, quite frankly, mentions of c-type lectin receptors were discussed in just a small branch of immunology. Now C-type lectins, especially dectin-1 (and now dectin-*2, how many others?*!) receptors are recognized as critically important in generating an inflammatory response to beta-glucans. Even more important, the response of C-type lectins recognizing glycoproteins, remains critical to understanding how low-dose erythropoietin (epo) reverses the ongoing activation of production of the short-lived anaphylatoxin, C4a. Low dose treatment with erythropoietin lowers C4a and stops its regeneration as described in two papers published by the Center for Research on Biotoxin Associated Illnesses (CRBAI: CDC conference on Chronic Fatigue Syndrome, 1/07, and American Society for Tropical Medicine and Hygiene, ASTMH, 11/07).

Summary

There are hundreds of academic citations and the overwhelming unity of agency and impartial opinion to support the concepts (1) that WDB host inflammagens and toxigens that are inhaled as the initiating event in the acquisition of illness; (2) that exposure to inhaled, not ingested, inflammagens and toxigens creates predictable human host responses; (3) that the human illness seen following inhalant exposure has epidemiologic consistency among multiple studies published in multiple diverse locations throughout the world; (4) that the inflammatory responses seen following inhalant exposure in affected patients are epidemiologically consistent and are mirrored by experimental data seen in vitro, in humans and in animals; (5) that treatment of affected patients is shown to be effective in peer-reviewed publications, including double-blinded, placebo-controlled clinical trials, and is further supported by experience of treating physicians, **a group whose experience, skill and observations have been omitted to date in the discussion regarding CIRS-WDB**; (6) that re-exposure of previously treated patients re-creates the illness within three days with an accentuation of (i) the ***magnitude*** of inflammatory host responses and (ii) the ***rate*** at which those enhanced inflammatory host responses occurs and; (7) prevention of illness by use of replacement doses of VIP (see treatment protocols) restores control of multiple physiologic abnormalities seen routinely in CIRS-WDB cases.

The reality is that there are no acceptable academic research papers that refute the presence of definable CIRS-WDB. The ACOEM statement is nearly universally referenced by subsequent defense industry-friendly publications without attempts to discuss the unscientific process used by the authors of ACOEM. The only experimental study on mold illness that Nay-Sayers can quote regarding long term effects of mold exposure is the single, high dose rat study that is specifically rejected by the rat study authors themselves from use in long term illness projections.

Finally, let us not forget the data supporting the comments in this report are generated from actual diagnosis and actual treatment of thousands of real, living patients. In contrast, assertions put forth by Nay-Sayers were based upon inaccurate assumptions made by consensus panels comprised of professionals not actually in clinical practice in the field of WDB illness. Our physicians aren't performing isolated experiments in rats, they are treating people. Our physicians aren't guessing what the illness might involve; they are following the established process of science using prospective exposures that give us the right to use the term, "caused," as validated by the peer-reviewers. Over 50,000 patients from 14 countries have been reported in peer-reviewed scientific articles. That is compelling evidence for the existence of CIRS-WDB. Any "academic statement" which rejects this truth, while simultaneously not providing <u>any</u> human physiologic data, should be considered to be irrelevant.

Epidemiology: Association, Causation, Bias, Confounders

Assessment of quality of epidemiologic papers has been well studied by epidemiologists, particularly with regard to study design and methods. Study design that is not based on prospective observations will not give us risk, which is integral to assessing causation. Studies of cases and controls give us associations that permit us to compare populations at given time points. The associations of patients with WDB who have abnormalities of innate immune responses are shared by no other disease entities except for those illnesses caused by biologically produced neurotoxins including dinoflagellates, cyanobacteria and apicomplexans. Review of the methods of studies allows us to reject a given study as reliable or not simply based on study design. Pertinent references follow.

1. Weed DL. Theory and practice in epidemiology. Ann NY Acad Sci 2001; 954: 52-62.

This 2001 overview of theory and practice in epidemiology provides the basis to understand that rigorous science is the first step toward applying theory in epidemiology.

2. Armstrong D. "Controversies in epidemiology," teaching causality in context at the University at Albany, School of Public Health. Scand J Public Health 1999; 27(2): 81-84.

Epidemiologic debates regarding causation in which there is demand to satisfy "Hill's Causal Criteria," require fundamental assumptions. When conceptualization of disease processes is restricted to conform to a causal paradigm, including mono-causality, there will be limitations on validity of such arguments.

3. Ebrahim S, Davey Smith G. Mendelian randomization: can genetic epidemiology help redress the failures of observational epidemiology? Hum Genet 2008; 123(1): 15-33.

A defect in the assignment of causation remains a reliance on observational studies. Establishing causal relationships between environmental exposures in common diseases is therefore fraught with errors of confounding, reverse causation and selection bias. Using Mendelian randomization, a functional genetic variant acts as a proxy for environmental exposure helping to support or reject causal hypothesis linking environmental exposure to common diseases. In this respect, use of HLA testing supports Mendelian randomization looking at cases and controls prospectively.

4. Lawlor DA, Harbord RM, Sterne JA, Timpson N, Davey Smith G. Mendelian randomization: Using genes as instruments for making causal inferences in epidemiology. Stat Med 2007; [Epub ahead of print].

This 2007 paper from the statistics literature reviews the inherent biases found in observational studies. Use of Mendelian randomization may help remove some of these built-in biases.

5. Nitsch D, Molokhia M, Smeeth L, DeStavola BL, Whittaker JC, Leon DA. Limits to causal inference based on Mendelian randomization: a comparison with randomized controlled trials. Am J Epidemiol 2006; 163(5): 397-403.

While Mendelian randomization is a powerful research tool, care in using it must be taken because drawing valid causal inferences from its applications also depends on existence of extensive assumptions.

6. Hernan MA. A definition of causal effect for epidemiological research. J Epidemiol Community Health 2004; 58(4): 265-271.

This paper is a classic in discussion of causal affect in epidemiology. Causation is discussed looking at dichotomous variables with the annulling assumption that no random error attributable to sampling variability exists. This paper presents the concept of causal theory. The limitations on randomized studies are reasons why methods for causal inference from observational data are needed. Contrast this proviso to that we see in prospective interventional trials in which using the same dichotomous variables gives the ability to conclude risk and therefore causation.

7. Hernan MA, Robins JM. Estimating causal effects from epidemiological data. J Epidemiol Community Health 2006; 60(7): 578-586.

If an experimental study design involves pure randomization then association is causation. The power of randomization in this case ensures that the exposed and non-exposed patients are interchangeable. In observational studies association is generally not causation because the exposed and unexposed are generally not exchangeable. The author is able to provide an estimate of casual affects from observational data requiring that description is restricted to dichotomous variables without random error attributable to sampling variability. In the studies of mold illness while involving treatment trials in which the patient serves as his own control and the exposure to a given environment remains constant. We have satisfaction of the demands from the author in that exposed and unexposed are exchangeable; the main description is restricted to dichotomous variables; and no random error of trivial sampling variability exist when patients are returning to the same environment previously noted to have adverse affects.

8. Petersen ML, Sinisi SE, van der Laan MJ. Estimation of direct causal effects. Epidemiology 2006; 17(3): 276-284.

Peterson’s estimation of direct effects is typical of the goal of research aimed at understanding pathways by which exposure acts to cause disease. Statistical methods used to estimate direct effects include logistic regression and a counter factual framework. These estimations are rendered moot if there is a prospective interventional study designed that provides for direct recording of objective measurements. Such a record coincides with time of exposure, time of intervention and time of conclusion of the intervention.

9. Little RJ, Rubin DB. Causal effects in clinical and epidemiological studies via potential outcomes: concepts and analytical approaches. Ann Rev Public Health 2000; 21: 121-145.

A problem in public health studies is how to make inferences about the causal effects of treatments. Without prospective inferential study design there will only be the opportunity to make inferences using statistical methods.

10. Kaufman JS, Cooper RS, Seeking causal explanations in social epidemiology. Am J Epidemiol 1999; 150(2): 113-120.

Causal explanations in social epidemiology pose particular problems for data analysis. Randomization is not possible; epidemiologic contrasts can not be derived; inferences become equivocal at best. Infectious disease epidemiology provides an example of approach to causal inference that can be used. This epidemiology is based on prospective documentation of risk from exposure.

11. Hofler M. Causal inference based on counterfactuals. BMC Med Res Methodol 2005; 5: 28.

Counterfactual modeling has become the basis of causal inference in medicine and epidemiology. Observational studies require such use; prospective documentation of acquisition of illness eliminates the need for epidemiologic inferences, including counterfactuals.

12. Robins JM, Greenland S. Identifiability and exchangeability for direct and indirect effects. Epidemiology 1992; 3(2): 143-155.

The difficulty of assessment of causation can involve separating direct effects of an exposure from intermediate or indirect effects of that exposure. If the exposure is randomized, direct or indirect effects are not separately identifiable. If the exposure is not randomized but is controlled, direct and indirect effects can be assessed without bias.

13. Kaufman JS, Maclehose RF, Kaufman S. A further critique of the analytic strategy of adjusting for covariates to identify biologic mediation. Epidemiol Perspect Innov 2004; 1(1): 4.

Studies devoted to investigation of causation that involve mechanistic inferences remain attractive aspects of epidemiologic research. These techniques may rely on assumptions which then make the biologic inference tenuous. The concepts of this paper need to be applied to any animal study done using a short term exposure and then extrapolating any of those results to chronic low dose exposure in humans. An assumption of monotonic affects is not supported by the inflammatory cascades seen in biological systems that employ amplifying innate immune response cascades.

14. Cole SR, Hernan MA. Fallibility in estimating direct effects. Int J Epidemiol 2002; 31(1): 163-165.

Estimating direct effects can be fraught with error if assumptions are made regarding unmeasured confounders for the causal effect of the mediator on the outcome. This paper supports absence of any extrapolation of effects from a one-time study in animals to long term affects in humans.

15. Klungel OH, Martens EP, Psaty BM, Grobbee DE, Sullivan SD, Stricker BH, Leufkens HG, de Boer A. Methods to assess intended effects of drug treatment in observational studies are reviewed. J Clin Epidemiol 2004; 57(12): 1223-1231.

Assumptions about unmeasured confounders remain the fundamental flaw of observational studies. More complete empirical evaluations comparing observational methods in different situations are needed.

16. Greenland S, Morgenstern H. Confounding in health research. Annu Rev Public Health 2001; 22: 189-212.

Consideration of confounding is fundamental to the design, analysis and interpretation of studies intended to estimate causal effects. This paper provides an overview to what confounding is. Use of a time-restricted prospective exposure model avoids potential confounders in all aspects of study design analysis and interpretation.

17. Klein-Geltink JE, Rochon PA, Dyer S, Laxer M, Anderson GM. Readers should systematically assess methods used to identify, measure and analyze confounding in observational cohort studies. J Clin Epidemiol 2007; 60(8): 766-772.

This 2007 paper emphasizes that readers should systematically assess methods used to identify measure- and analysis-confounding in observational cohort studies. Studies looking at acquisition of illness in humans must include adequately matched cases and controls such that they are interchangeable; accurate recordation of symptoms and correlation with objective laboratory studies. Addition of genomic studies is of tremendous importance.

18. Levin NW, Handelman GJ, Coresh J, Port FK, Kaysen GA. Reverse epidemiology: a confusing, confounding, and inaccurate term. Semin Dial 2007; 20(6): 586-592.

The renal dialysis literature contains an interesting twist on classical epidemiology. “Reverse epidemiology,” looks at assumptions made regarding health and populations that are reversed in their findings in people with renal failure and dialysis. This paper simply supports the need to look at projective parameters prospectively without assumptions.

19. Blair A, Stewart P, Lubin JH, Forastiere F. Methodological issues regarding confounding and exposure misclassification in epidemiological studies of occupational exposures. Am J Ind Med 2007; 50(3): 199-207.

The exposure misclassification i.e. assigning a case to a control and a control to be a case, will limit interpretation of any retrospective epidemiologic studies.

20. Fewell Z, Davey Smith G, Sterne JA. The impact of residual and unmeasured confounding in epidemiologic studies: a simulation study. Am J Epidemiol 2007; 166(6): 646-655.

Measurement error in explanatory variables and unmeasured confounders cause considerable problems in epidemiologic studies. Any assumptions made in epidemiologic

studies must be plausible. An example of an implausible study gives the assumption that a one-time exposure of rats to spores can be extrapolated to human health effects with chronic low dose exposure to a multi-factorial environment found in water-damaged buildings. Note the applicability this is referring to the Rao study cited to support the authors' conclusions in the ACOEM study extrapolating from rodents to humans to claim that no toxic effect was possible, it must be remembered that the spores used in this study had been washed in methanol, thereby potentially removing unknown quantities of toxins before they were administered to the animals. This process insured an effect of exposure that is reduced by an unknown factor. Given the serious illness induced by the attenuated spores, one can see why parallel studies done by Dr. Thomas Rand (2003) using ***one tenth*** the dose of unwashed spores caused massive pulmonary damage to the studied animals.)

21. MacLehose RF, Kaufman JS, Poole C. Bounding causal effects under uncontrolled confounding using counterfactuals. Epidemiology 2005; 16(4): 548-555.

Counterfactual analysis can assist when there is uncontrolled confounding in studies. Use of a prospective interventional study design can eliminate the need for these theoretical discussions.

22. Chyou PH. Patterns of bias due to differential misclassification by case-control status in a case-control study. Eur J Epidemiol 2007; 22(1): 7-17.

Bias can be introduced into a case control study by misclassification of patients. Use of a clinical trial with a study design called ABB`AB avoids differential misclassification.

23. Correa-Villasenor A, Stewart WF, Franco-Marina F, Seacat H. Bias from nondifferential misclassification in case-control studies with three exposure levels. Epidemiology 1995; 6(3): 276-281.

Case controls studies using multiple exposure levels where disease risk is associated with exposure demands accurate classification of patients.

24. Greenland S, Gustafson P. Accounting for independent nondifferential misclassification does not increase certainty that observed association is in the correct direction. Am J Epidemiol 2006; 164(1): 63-68.

Exposure measurement errors are not independent of other errors; they lead to study bias. Use of a 2 x 2 table, looking at dichotomous variable, avoids some of this non-differential misclassification and reduces bias.

25. Brenner H. Inferences on the potential effects of presumed nondifferential exposure misclassification. Ann Epidemiol 1993; 3(3): 289-294.

Non-differential exposure misclassification leads to the potential for false inferences based on unsupported assumptions.

26. Delgado-Rodriguez M, Llorca J. Bias. J Epidemiol Community Health 2004; 58(8): 635-641.

Bias can be defined as a lack of internal validity for an incorrect assessment of association between and exposure and an effect. Bias may be introduced by definition and selection of study population; data collection; and association between different determinates of an effect in the population.

27. Mark DH. Interpreting the term selection bias in medical research. Fam Med 1997; 29(2): 132-136.

Selection bias is often poorly understood. Looking at selection in terms of representative subjects, exposures and selection of subject of outcomes must contain avoidance of bias. Study findings may be distorted if selection into the study is according to exposure status.

28. Hernan MA, Hernandez-Diaz S, Robins JM. A structural approach to selection bias. Epidemiology 2004; 15(5): 615-625.

Selection bias encompasses various biases in epidemiology. Restriction of information and inappropriate selection controls are two examples of selection bias. This paper is an organized theoretical basis of selection bias. Use of the patient as his own control will eliminate selection bias.

29. Bateson TF, Schwartz J. Selection bias and confounding in case-crossover analyses of environmental time-series data. Epidemiology 2001; 12(6): 654-661.

Selection bias results when exposure in the reference period is not identical to exposure in the "hazard." In this case, the exposure must be controlled, with time as a critical element supporting such uniformity of exposure. If exposure is identical then elements of confounders that could contribute to selection bias can be reduced by using shorter reference spacing lengths. This is exactly the reason that our intervention studies are time-limited.

30. Pearce N, Checkoway H, Kriebel D. Bias in occupational epidemiology studies. Occup Environ Med 2007; 64(8): 562-568.

Occupational epidemiology studies contain significant potential for bias including selection bias, information bias and the role of confounding. Selection bias can be minimized by obtaining high response rates. Information bias is minimized by avoiding elements such as questionnaires. Confounding of exposures demands that exposure remains constant. Our interventional studies follow these principles, eliminating bias.

31. Westhoff CL. Epidemiologic studies: pitfalls in interpretation. Dialogues Contracept 1995; 4(5): 5-6, 8.

This overview paper looks at proper design of epidemiologic studies. Careful selection of control groups, elimination of selection bias, diagnostic suspicion bias, and susceptibility bias are keys. Elimination of two or more simultaneous exposures eliminates those confounders. Control for age and disease state also is important.

32. Joffe MM, Colditz GA. Restriction as a method for reducing bias in the estimation of direct effects. Stat Med 1998; 17(19): 2233-2249.

This paper from the statistical literature looks at measurement of directs effects of treatment on outcome. This paper is more directed at observational studies than on prospective interventional studies.

33. Wunsch H, Linde-Zwirble WT, Angus DC. Methods to adjust for bias and confounding in critical care health services research involving observational data. J Crit Care 2006; 21(1): 1-7.

This paper also summarizes the inherent bias built into observational studies. Looking at potential sources of bias and confounding is necessary.

34. Navidi W, Thomas D, Langholz B, Stram D. Statistical methods for epidemiologic studies of the health effects of air pollution. Res Rep Health Eff Inst 1999; (86): 1-50.

Epidemiologic studies involving environmental exposure must have accurate information about past exposure and ensure the level exposure remains unaffected by the response of the subject.

35. Li Y, Sung FC. A review of the healthy worker effect in occupational epidemiology. Occup Med (Lond) 1999; 49(4): 225-229.

The concept of “healthy worker effect” must be addressed as a source of confounding bias. Looking at all employees of a manufacturing facility for example, would not include the bias introduced by exclusion of unhealthy workers before employment and that the study of the active workers in as health workers would not be necessarily applicable to that of the entire population.

36. Baillargeon J, Wilkinson G, Rudkin L, Baillargeon G, Ray L. Characteristics of the healthy worker effect: a comparison of male and female occupational cohorts. J Occup Environ Med 1998; 40(4): 368-373.

The healthy worker effect must include controls on age, gender, race, occupational class, and length of follow-up.

37. Meijers JM, Swaen GM, Volovics A, Lucas LJ, van Vliet K. Occupational cohort studies: the influence of design characteristics on the healthy worker effect. Int J Epidemiol 1989; 18(4): 970-975.

Occupational cohort studies that use retrospective design have a massive healthy worker effect built in. These studies should be avoided.

Cognitive Deficits and Inflammation

1. Wilson CJ, Finch CE, Cohen HJ. Cytokines and cognition-the case for a head-to-toe inflammatory paradigm. J Am Geriatr Soc 2002; 50(12): 2041-2056.

The brain is immunologically unique; neuroimmunoendocrine processes are subject to peripheral influences from inflammatory mediators including cytokines. Cytokines contribute to cognitive impairment via cytokine-mediated interactions between neurons and glial cells. Cytokines affect cellular mechanisms subserving cognition (cholinergic and dopaminergic pathways). Cytokines play a key role in the hypothalamic-pituitary-adrenal axis that participates in symptoms from stress and depression.

2. Eskandari F, Webster JI, Sternberg EM. Neural immune pathways and their connection to inflammatory diseases. Arthritis Res Ther 2003; 5(6): 251-265.

In the central nervous system inflammation and inflammatory responses are modulated by an interaction and communication between the neuroendocrine system and the immune system. Cytokines cross the blood brain barrier, signal the CNS through vagal nerve pathways and second messengers including (cAMP).

3. Hopkins SJ. Central system recognition of peripheral inflammation: a neural, hormonal collaboration. Acta Biomed 2007; 78 Suppl 1: 231-247.

Cytokines and lipopolysaccharides induce production of prostaglandins which affect neural pathways and CNS responses. Interleukin-6 readily gains access to the CNS and acts by inducing changes in brain microvasculature which in turn alters activity of neurons controlling multiple bodily functions including behavioral responses.

4. Watkins LR, Maier SF. Immune regulation of central nervous system functions: from sickness responses to pathological. J Intern Med 2005; 257(2): 139-155.

The brain modulates immune responses; immune responses modulate brain activity. Particular inflammatory pathways called “sickness responses” are created by activating glial cells in the CNS to release pro-inflammatory cytokines. This mechanism is now shown to enhance pain responsivity. This paper looks at pathological chronic pain that results from this ancient CNS circuitry.

5. Maier SF, Watkins LR. Immune-to-central nervous system communication and its role in modulating pain and cognition: Implications for cancer and cancer treatment. Brain Behav Immun 2003; 17 Suppl 1: S125-131.

Pro-inflammatory cytokines released by immune cells signal the brain by both blood-borne and neural roots leading to alterations in neural activity. Cytokines in the brain, specifically in the hippocampus, interfere with consolidation of memory; cytokines within the spinal cord exaggerate pain.

6. Banks WA, Farr SA, Morley JE. Entry of blood-borne cytokines into the central nervous system: effects on cognitive processes. Neuroimmunomodulation 2002-2003; 10(6): 319-327.

An important mechanism for delivery of cytokines from peripheral blood circulation to the brain is transporting cytokines across the blood brain barrier. The transport of cytokines induces impairment in memory and sickness behavior.

7. Viljoen M, Koorts AM. A role for proinflammatory cytokines in the behavioral disturbances and cognitive decline in chronic renal failure patients. Clin Nephrol 2004; 61(3): 227-229.

Abstract not presented.

8. Wolfe F, Michaud K. Fatigue, rheumatoid arthritis, and anti-tumor necrosis factor therapy: an investigation in 24,831 patients. J Rheumatol 2004; 31(11): 2115-2120.

In a study of over 24,000 patients with rheumatoid arthritis, symptoms were ranked showing that loss of function, chronic pain, depression and fatigue dominated. Anti-TNF therapy improves other symptoms but fatigue was not affected significantly.

9. Yaffe K, Kanaya A, Lindquist K, Simonsick EM, Harris T, Shorr RI, Tylavsky FA, Newman AB. The metabolic syndrome, inflammation, and risk of cognitive decline. JAMA 2004; 292(18): 2237-2242.

This important paper from JAMA showed a statistically significant effect of inflammation on cognitive impairment. Those with high inflammation had an increased likelihood of cognitive impairment compared to those without. This study supports a hypothesis that inflammatory events contribute to cognitive impairment.

10. Tonelli LH, Postolache TT. Tumor necrosis factor alpha, interleukin-1 beta, interleukin-6 and major histocompatibility complex molecules in the normal brain and after peripheral immune challenge. Neurol Res 2005; 27(7): 679-684.

This paper from the University of Maryland shows the production of cytokines in response to an immune challenge in the periphery has been well established to activate an inflammatory response in the brain. Important cytokines causing changes in brain function are IL-1β, TNF and interleukin-6. Class I major histocompatibility complex (HLA) molecules are up-regulated in the brain in response to peripheral administration of bacterial products. Mechanisms of defense against pathogens can dramatically affect brain structure and function inducing changes in cognition, mood and behavior. Other inflammatory challenges may trigger worsening of previous functional deficits.

11. Tonelli LH, Postolache TT, Sternberg EM. Inflammatory genes and neural activity: involvement of immune genes in synaptic function and behavior. Front Biosci 2005; 10: 675-680.

Dysregulation of immune genes and cerebral spinal fluid of patients with psychiatric disorders have a role in mood and cognition. This known effect of inflammation is mediated through cytokines and HLA.

12. Licinio J, Kling MA, Hauser P. Cytokines and brain function: relevance to interferon-alpha-induced mood and cognitive changes. Semin Oncol 1998; 25(1 Suppl 1): 30-38.

Treatment with INF-α can adversely affect mood and cognition causing depression, memory disturbance and mood disorders.

13. Capuron L, Miller AH. Cytokines and psychopathology: lessons from interferon-alpha. Biol Psychiatry 2004; 56(11): 819-824.

INF-α treated patients show at least two distinct syndromes. 1) a mood cognitive syndrome that responds to anti-depressants. 2) a neuro-vegetative syndrome characterized by psychomotor slowing and fatigue that does not respond to anti-depressants.

14. Owens T, Babcock A. Immune response induction in the central nervous system. Front Biosci 2002; 7: d427-438.

The central nervous system is noted for its "immune privilege." There are inflammatory effects in the brain that cause symptoms following peripheral infection and peripheral inflammation.

15. Chavarria A, Alcocer-Varela J. Is damage in central nervous system due to inflammation? Autoimmun Rev 2004; 3(4): 251-260.

Central nervous system inflammation can have an initial reparative role following immune activation. Over time, neuroinflammation becomes damaging.

16. Millward JM, Caruso M, Campbell IL, Gauldie J, Owens T. IFN-gamma-induced chemokines synergize with pertussis toxin to promote T cell entry to the central nervous system. J Immunol 2007; 178(12): 8175-8182.

This 2007 paper highlights the synergistic interaction of INF-γ with the pertussis toxin to increase T-cell entry into the central nervous system. The T-cell infiltration is greater than with treatment by toxin or INF-γ alone.

17. Hagberg H, Mallard C. Effect of inflammation on central nervous system development and vulnerability. Curr Opin Neurol 2005; 18(2): 117-123.

The effects of inflammation on central nervous system development in children may have severe consequences for the individual that persist from childhood into adulthood.

18. Magaki S, Mueller C, Dickson C, Kirsch W. Increased production of inflammatory cytokines in mild cognitive impairment. Exp Gerontol 2007; 42(3): 233-240.

Cytokines made peripherally have effects on cognition.

19. Lindberg C, Chromek M, Ahrengart L, Brauner A, Schultzberg M, Garlind A. Soluble interleukin-1 receptor type II, IL-18 and caspase-1 in mild cognitive impairment and severe Alzheimer's disease. Neurochem Int 2005; 46(7): 551-557.

This 2007 paper looks at the pathogenic role for chronic inflammation initiated peripherally creating central nervous system effects, including cognitive impairment. In patients with mild cognitive impairment who underwent spinal fluid testing, elevated levels of interleukin-1 receptor and interleukin-18 were found.

20. Dik MG, Jonker C, Hack CE, Smit JH, Comijs HC, Eikelenboom P. Serum inflammatory proteins and cognitive decline in older persons. Neurology 2005; 64(8): 1371-1377.

The highest level of an inflammatory protein found in blood was associated with the most increased risk of decline on a test of cognition.

21. Boutin H, LeFeuvre RA, Horai R, Asano M, Iwakura Y, Rothwell NJ. Role of IL-1α and IL-1β in ischemic brain damage. J Neurosci 2001; 21(15): 5528-5534.

IL-1 plays a major role in subsequent injury to the brain following an injury to the brain from ischemia (lack of oxygen).

22. Zhu Y, Saito K, Murakami Y, Asano M, Iwakura Y, Seishima M. Early increase in mRNA levels of pro-inflammatory cytokines and their interactions in the mouse hippocampus after transient global ischemia. Neurosci Lett 2006; 393(2-3): 122-126.

Transient reduction of oxygen delivery into the hippocampus of a mouse is associated with a significant rise in mRNA levels of pro-inflammatory cytokines. Therefore, capillary hypoperfusion will be greeted with an inflammatory response in the brain.

23. Sheng WS, Hu S, Ding JM, Chao CC, Peterson PK. Cytokine expression in the mouse brain in response to immune activation by *Corynebacterium parvum*. Clin Diagn Lab Immunol 2001; 8(2): 446-448.

Injection of *Corynebacteria* into the abdominal cavity of mice was followed by a prolonged upregulation of cytokine expression in the brain and subcorticol structures of the brain.

24. Gelinas DS, McLaurin J. PPAR-alpha expression inversely correlates with inflammatory cytokines IL-1β and TNF-α in aging rats. Neurochem Res 2005; 30(11): 1369-1375.

There is an age-dependent increase of expression of IL-1β and TNF in the brains of rats. Increasing PPAR expression results in beneficial effects on cognition and reduction of cytokine expression.

25. Montalban X, Rio J. Interferons and cognition. J Neurol Sci 2006; 245(1-2): 137-140.

This 2006 paper from Spain shows that there is an influence of INF β on neuropsychological aspects of brain activity in patients with multiple sclerosis.

26. Pierson SH, Griffith N. Treatment of cognitive impairment in multiple sclerosis. Behav Neurol 2006; 17(1): 53-67.

Cognitive impairment in multiple sclerosis is increasingly recognized. The therapeutic interventions including interferons that can assist in reduction of the cognitive impairment are discussed.

27. Magaki S, Mueller C, Dickson C, Kirsch W. Increased production of inflammatory cytokines in mild cognitive impairment. Exp Gerontol 2007; 42(3): 233-240.

Inflammation and cognitive impairment are linked.

28. Wan Y, Xu J, Ma D, Zeng Y, Cibelli M, Maze M. Postoperative impairment of cognitive function in rats: a possible role for cytokine-mediated inflammation in the hippocampus. Anesthesiology 2007; 106(3): 436-443.

This 2007 paper looks at the affect of cytokine mediated inflammation in the hippocampus as a possible source for impairment of cognitive function in rats observed post-operatively. Cognitive decline is associated with a hippocampal inflammatory response.

29. Rafnsson SB, Deary IJ, Smith FB, Whiteman MC, Rumley A, Lowe GD, Fowkes FG. Cognitive decline and markers of inflammation and hemostasis: the Edinburgh Artery Study. J Am Geriatr Soc 2007; 55(5): 700-707.

This paper from the Edinburgh Study (similar to the Framingham study in the US) shows that systemic markers of inflammation are associated with progressive decline in general and specific cognitive abilities in older people independent of changes in blood vessels.

30. Dehghani F, Conrad A, Kohl A, Korf HW, Hailer NP. Clodronate inhibits the secretion of proinflammatory cytokines and NO by isolated microglial cells and reduces the number of proliferating glial cells in excitotoxically injured organotypic hippocampal slice cultures. Exp Neurol 2004; 189(2): 241-251.

Clodronate can inhibit microglial secretion of pro-inflammatory cytokines and nitric oxide. This drug could prove to be a useful tool in the investigation of interactions between damaged neurons and microglial cells. It shows potential for use in patients with cognitive decline associated with inflammatory changes in the brain.

31. Hailer NP, Vogt C, Korf HW, Dehghani F. Interleukin-1β exacerbates and interleukin-1 receptor antagonist attenuates neuronal injury and microglial activation after excitotoxic damage in organotypic hippocampal slice cultures. Eur J Neurosci 2005; 21(9): 2347-2360.

IL-1B directly affects the CNS inducing microglial activation without neurotoxicity and enhancing injury. It enhances excitotoxic neuronal damage. Protection from damage is

obtained by use of IL-1 receptor antagonists. Protection from damage cognitive decline and inflammatory conditions with anti-inflammatory medications remains of interest.

32. Rothwell N. Interleukin-1 and neuronal injury: mechanisms, modification, and therapeutic potential. Brain Behav Immun 2003; 17(3): 152-157.

IL-1 expression in the brain can contribute directly to ischaemic excitotoxic and traumatic brain damage. The implication is that use of interleukin-1 receptor antagonist could assist in treatment of stroke and cognitive disorders.

33. Simi A, Tsakiri N, Wang P, Rothwell NJ. Interleukin-1 and inflammatory neurodegeneration. Biochem Soc Trans 2007; 35(Pt 5): 1122-1126.

Interleukin-1 is expressed rapidly in response to neuronal injury, most prominently by microglial cells. Elevated levels of endogenous or exogenous interleukin-1 worsen injury. Protection is obtained from interleukin-1 receptor antagonists.

34. Lucas SM, Rothwell NJ, Gibson RM. The role of inflammation in CNS injury and disease. Br J Pharmacol 2006; 147 Suppl 1: S232-240.

There is much evidence showing that inflammation and inflammatory mediators contribute to acute, chronic and psychiatric central nervous system disorders. There is a complex interplay between inflammatory mediators, aging, genetic background in environmental factors.

35. Clarkson AN, Rahman R, Appleton I. Inflammation and autoimmunity as a central theme in neurodegenerative disorders: fact or fiction? Curr Opin Investig Drugs 2004; 5(7): 706-713.

While inflammation is recognized as having a central role in multiple sclerosis, Alzheimer's disease, Parkinson's disease, simple treatment of cytokine responses alone is unlikely to be successful in treating neurodegenerative diseases.

36. Cunningham C, Wilcockson DC, Campion S, Lunnon K, Perry VH. Central and systemic endotoxin challenges exacerbate the local inflammatory response and increase neuronal death during chronic neurodegeneration. J Neurosci 2005; 25(40): 9275-9284.

Systemic endotoxin presence, using lipopolysaccharide, dramatically increases interleukin-1 beta expression, infiltration of neutrophil and nitric oxide production in the brain. Central and peripheral inflammation can exacerbate brain inflammation in neuronal injury. A single acute systemic inflammatory event can induce neuronal changes in the central nervous system.

37. Perry VH. The influence of systemic inflammation on inflammation in the brain: implications for chronic neurodegenerative disease. Brain Behav Immun 2004; 18(5): 407-413.

Systemic inflammation is passed into the brain by cytokines and macrophages. The effect of systemic inflammation on local inflammation in the brain leads to an exaggerated synthesis of inflammatory cytokines in the brain, which in turn influences behavior.

38. Schultzberg M, Lindberg C, Aronsson AF, Hijorth E, Spulber SD, Oprica M. Inflammation in the nervous system-physiological and pathophysiological aspects. Physiol Behav 2007; 92(1-2): 121-128.

This 2007 paper from the Karolinska Institute focuses on the effect on cytokines, especially the interleukin-1 system on neurodegeneration, cognition and temperature changes.

39. Ehrenreich H, Fischer B, Norra C, Schellenberger F, Stender N, Stiefel M, Siren AL, Paulis W, Nave KA, Gold R, Bartels C. Exploring recombinant human erythropoietin in chronic progressive multiple sclerosis. Brain 2007; 130(Pt 10): 2577-2588.

This 2007 paper shows benefit from use of erythropoietin in treatment of chronic progressive multiple sclerosis. The dose of epo used was 48,000 units given once a week. Note: in our IRB-approved studies we use erythropoietin in lower doses twice a week with significant increase in benefit compared to once a week. The half life of epo is approximately 1 ½ days. Once weekly dosing is illogical.

40. Guerreiro RJ, Santana I, Bras JM, Santiago B, Paiva A, Oliveira C. Peripheral inflammatory cytokines as biomarkers in Alzheimer's disease and mild cognitive impairment. Neurodegener Dis 2007; 4(6): 406-412.

This 2007 paper supports the findings from studies showing that persistent inflammatory status leads to progressive impairment in cognition.

41. Robinson EK, Seaworth CM, Suliburk JW, Adams SD, Kao LS, Mercer DW. Effect of NOS inhibition on rat gastric matrix metalloproteinase production during endotoxemia. Shock 2006; 25(5): 507-514.

In clinical conditions where endotoxin is present in the blood there is a significant increase in production of metalloproteinases (MMPs). These MMPs can delivery inflammatory elements out of blood into target tissues including brain. Exposure to endotoxin from water-damaged buildings will have the same effect.

42. Suliburk JW, Helmer KS, Kennison SD, Mercer DW, Robinson EK. Time-dependent aggravation or attenuation of lipopolysaccharide-induced gastric injury by nitric oxide synthase inhibition. J Surg Res 2005; 129(2): 265-271.

During presence of endotoxin in the blood there is a series of inflammatory events that affect GI motility as well as nitric oxide production.

43. de la Torre JC, Aliev G. Inhibition of vascular nitric oxide after rat chronic brain hypoperfusion: spatial memory and immunocytochemical changes. J Cereb Blood Flow Metab 2005; 25(6): 663-672.

Blockade of nitric oxide following capillary hypoperfusion in the brain contributed to marked worsening in performance on maze testing in rats. The role of nitric oxide in spatial memory function is to keep cerebral perfusion optimal. This optimal perfusion is reduced by metalloproteinase activity.

44. Institoris A, Farkas E, Berczi S, Sule Z, Bari F. Effects of cyclo-oxygenases (COX) inhibition on memory impairment and hippocampal damage in the early period of cerebral hypoperfusion in rats. Eur J Pharmacol 2007; 574(1): 29-38.

Capillary hypoperfusion in hippocampus results in learning impairment, mild neuronal damage, injury to dendrites and moderate activation of astrocytes in the hippocampus. This hypoperfusion-induced memory impairment can be blocked experimentally by the drug, NS-398.

45. Shibata M, Yamasaki N, Miyakawa T, Kalaria RN, Fujita Y, Ohtani R, Ihara M, Takahashi R, Tomimoto H. Selective impairment of working memory in a mouse model of chronic cerebral hypoperfusion. Stroke 2007; 38(10): 2826-2832.

In mice with chronic cerebral capillary hypoperfusion there is selective impairment of working memory found without clear lesions in the hippocampus. The memory deficit is attributed to inflammatory damage to frontal lobes.

46. Zhou YF, Stabile E, Walker J, Shou M, Baffour R, Yu Z, Rott D, Yancopoulos GD, Rudge JS, Epstein SE. Effects of gene delivery on collateral development in chronic hypoperfusion: diverse effects of angiopoietin-1 versus vascular endothelial growth factor. J Am Coll Cardiol 2004; 44(4): 897-903.

With persistent hypoperfusion, there is activation of VEGF to compensate for tissue hypoxia. Over-expression of VEGF, after causing an inflammatory response of its own, does not improve collateral flow.

47. Moe CL, Turf E, Oldach D, Bell P, Hutton S, Savitz S, Koltai D, Turf M, Ingsrisawang L, Hart R, Ball JD, Stutts M, McCarter R, Wilson L, Haselow D, Grattan L, Morris JG, Weber DJ. Cohort studies of health effects among people exposed to estuarine waters: North Carolina, Virginia, and Maryland. Environ Health Perspect 2001; 109 Suppl 5: 781-786.

This paper from UNC and the University of Maryland is a follow-up of neuropsychological deficits noted in patients exposed to toxins made by *Pfiesteria.*

48. Hudnell HK, House D, Schmid J, Koltai D, Stopford W, Wilkins J, Savitz DA, Swinker M, Music S. Human visual function in the North Carolina clinical study on possible estuary-associated syndrome. J Toxicol Environ Health A 2001; 62(8): 575-594.

Visual contrast sensitivity deficits are demonstrated in patients exposed to fish kills and *Pfiesteria* in North Carolina. This VCS deficit was the only abnormality found in evaluation of these patients; having VCS deficits was predictive of neuropsychological impairment.

49. Qin L, Liu Y, Wang T, Wei SJ, Block ML, Wilson B, Liu B, Hong JS. NADPH oxidase mediates lipopolysaccharide-induced neurotoxicity and proinflammatory gene expression in activated microglia. J Biol Chem 2004; 279(2): 1415-1421.

Endotoxin and LPS can induce neurotoxicity in activation of genes producing pro-inflammatory mediators in glial cells.

50. Qin L, Wu X, Block ML, Liu Y, Breese GR, Hong JS, Knapp DJ, Crews FT. Systemic LPS causes chronic neuroinflammation and progressive neurodegeneration. Glia 2007; 55(5): 453-462.

Systemic lipopolysaccharide presence causes chronic inflammation and progressive degeneration in the brain.

51. Sparkman NL, Buchanan JB, Heyen JR, Chen J, Beverly JL, Johnson RW. Interleukin-6 facilitates lipopolysaccharide-induced disruption in working memory and expression of other pro-inflammatory cytokines in hippocampal neuronal cell layers. J Neurosci 2006; 26(42): 10709-10716.

This 2006 paper shows that an inflammatory cytokine, interleukin-6, increases disruption induced by lipopolysaccharide in memory by affecting hippocampus.

52. Huang Y, Henry CJ, Dantzer R, Johnson RW, Godbout JP. Exaggerated sickness behavior and brain pro-inflammatory cytokine expression in aged mice in response to intracerebroventricular lipopolysaccharide. Neurobiol Aging 2007; [Ebup ahead of print]

Direct administration of lipopolysaccharide into the brain creates inflammatory responses in the brain and activation of “prolonged sickness behavior,” that are worse in aged compared to adult mice.

53. Godbout JP, Chen J, Abraham J, Richwine AF, Berg BM, Kelley KW, Johnson RW. Exaggerated neuroinflammation and sickness behavior in aged mice following activation of the peripheral innate immune system. FASEB J 2005; 19(10): 1329-1331.

This paper shows that activation of peripheral innate immune system responses leads to exaggerated neuroinflammation in mice. This dysregulation between peripheral and central innate immune system is likely to be involved with severe behavioral deficits that occur frequently in adults with systemic infections.

54. Pereira C, Agostinho P, Moreira PI, Cardoso SM, Oliveira CR. Alzheimer’s disease-associated neurotoxic mechanisms and neuroprotective strategies. Curr Drug Targarets CNS Neurol Disord 2005; 4(4): 383-403.

This paper focuses on neurotoxicity in Alzheimer’s disease. This paper is included in this group to support the role of inflammation in Alzheimer’s together with cognitive and memory impairment found in that disease.

55. Ghavami A, Hirst WD, Novak TJ. Selective phosphodiesterase (PDE)-4 inhibitors: a novel approach to treating memory deficit? Drugs R D 2006; 7(2): 63-71.

Use of selective inhibitors of phosphodiesterase-4, drugs that can regulate intracellular levels of cyclic (cAMP), shows great promise in restoring memory deficits.

56. Chen J, Buchanan JB, Sparkman NL, Godbout JP, Freund GG, Johnson RW. Neuroinflammation and disruption in working memory in aged mice after acute stimulation of the peripheral innate immune system. Brain Behav Immun 2008; 22(3): 301-311.

This 2008 study shows that neurobehavioral complications are associated with peripheral infections, possibly by allowing the expression of inflammatory cytokines in brain areas that mediate cognitive processing. Hippocampal processing is more easily disrupted in older animals than younger animals. These disruptions are associated with inflammatory cytokines in the brain.

57. Zhou HR, Harkema JR, Yan D, Pestka JJ. Amplified pro-inflammatory cytokine expression and toxicity in mice co-exposed to lipopolysaccharide and the trichothecene vomitoxin (deoxynivalenol). J Toxicol Environ Health A 1999; 57(2): 115-136.

A trichothecene fungal toxin (DON) amplifies the pro-inflammatory cytokine response in mice exposed to lipopolysaccharide.

58. Lang CH, Silvis C, Deshpande N, Nystrom G, Frost RA. Endotoxin stimulates in vivo expression of inflammatory cytokines tumor necrosis factor alpha, interleukin-1beta, IL-6, and high-mobility-group protein-1 in skeletal muscle. Shock 2003; 19(6): 538-546.

Endotoxin in the blood stimulates expression of inflammatory cytokines peripherally. These cytokines cross the blood brain barrier.

59. Browne SE, Lin L, Mattsson A, Georgievska B, Isacson O. Selective antibody-induced cholinergic cell and synapse loss produce sustained hippocampal and cortical hypometabolism with correlated cognitive deficits. Exp Neurol 2001; 170(1): 36-47.

Reduction of metabolism in hippocampus and brain cortex is associated with cognitive deficits. Sustained reduction in glucose utilization in brain regions affected included frontal lobes and hippocampal areas. Impaired cognitive spatial performance in a maze test was noted in animals with reduced glucose use. These findings parallel those seen in patients with elevated central nervous system lactate levels seen on MR spectroscopy.

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Recovery from bacterial sepsis, a model for lipopolysaccharide and endotoxin exposure, shows loss of neuronal activity in hippocampus and pre-frontal cortex. Sepsis therefore can induce produce persistent behavioral neuro anatomical changes.

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An anti-inflammatory cytokine, IL-4, shows balance in the ability to stop abnormal effects of inflammatory responses in the brain.

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Interleukin-4 plays a central role in modulated expression of microglial cells.

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This 2007 paper supports previously referenced studies looking at activation of PPAR gamma with drugs (TZD) such as pioglitazone. The benefits of these drugs include improvement of cognitive deficits and mood disorders. This paper supports the hypothesis that TZD maybe salutary for cognitive deficits and associate mood disorder.

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This 2007 paper looks at acute systemic inflammation as well as inflammatory responses seen centrally and peripherally. There are changes in ability to remember, so called mnesic deficits. It is unknown if this damage to areas involved with memory and mitochondria in the brain are associated with neurodegenerative disease.

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Memantine can oppose the neuro-inflammation associated with exposure to lipopolysaccharides. By reducing neuroinflammation spatial learning is restored.

66. Ohta H, Nishikawa H, Kimura H, Anayama H, Miyamoto M. Chronic cerebral hypoperfusion by permanent internal carotid ligation produces learning impairment without brain damage in rats. Neuroscience 1997; 79(14): 1039-1050.

Cerebral hypoperfusion causes learning impairment without brain damage. Reduction of blood flow is an important factor that causes or exacerbates cognitive decline.

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Cerebral hypoperfusion can cause capillary damage in hippocampus that is associated and correlates with spatial memory impairment. Capillary integrity is one of the most important brain functions in conditions of compromised cerebral microcirculation. This finding is exactly what we have seen in our MR spectroscopy studies.

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An additional compound, paeoniflorin, shows promise to attenuate cognitive deficits in brain damage induced by chronic cerebral hypoperfusion. Suppression of neuroinflammation in the brain may be involved with neuro-protection.

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An increase in glial metabolites is associated with deficits in working memory. This same mechanism may contribute to cognitive function and other brain diseases that involve inflammation.

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The authors point out that ultrafine (nano-size particles) and fine particles with attached organic and inorganic compounds enter the systemic circulation via RBCs and reach the brain causing neuroinflammation. The inflammation results from the disruption of the blood brain barrier resulting in microglial activation and production of pro-inflammatory cytokines (IL1-β, TNFα, and INFγ). This excellent review is based upon observations on both animals (rodents and canines) and children and young adults exposed to air pollution.

Inflammation from WDB

Recent literature has confirmed that the sources of inflammatory responses seen in patients with illness acquired following exposure to the interior environments of water-damaged buildings (WDB) are multifactorial (GAO, AIHA *Recognition, Evaluation, and Control of Indoor Mold*, CDC/Rao 3/07), with inhalation the predominant mode of exposure. Taken as a whole, the inflammatory responses are those of innate immune responses, with abnormalities in innate immune responses creating adverse health effects. Because multiple possible sources of innate immune activation co-exist in the indoor environment of a water-damaged building (WDB), it is not always possible to determine which effector caused a specific immune response and in turn, which immune response generated a specific symptom.

Innate immune responses are initiated by pattern recognition of foreign antigens by specific receptors. Such pattern recognition will lead to release of pro-inflammatory cytokines, activation of complement and differential gene activation. Each of these multiple events in turn initiates an ever-expanding response, either from more activation of pre-formed elements of complement and more pro-inflammatory cytokines or from more differential gene activation that then leads to additional elements of inflammation (Th-1 responses). Each of the initial effectors of innate immune responses then leads to an additional, ever-expanding cascade of downstream inflammatory responses. Newer data show that an additional series of inflammatory pathway responses (Th-17) are mediated by TGF β-1. TGF β-1 in turn affects DNA replication; epithelial to mesenchymal transformation (primarily in lung but also in kidney and liver); and inhibition of normal regulatory T-lymphocyte function, thereby contributing to loss of recognition of self and increased development of autoantibodies.

If the process of antigen presentation, initiated by pattern recognition, leads to normal production of protective antibodies that clear the offending antigen(s), the process of innate immune response to foreign antigen ceases. The downstream pathway of heightened innate immune response and the health effects of the innate immune effectors end. If there is defective antigen presentation, with the potential that the process of antigen presentation could go awry in a number of intervening pathways; or if there is ongoing re-exposure (the patient is not removed from the interior environment of a WDB, for example) then the process of innate immune stimulation will not stop. If there is ongoing innate immune activation beginning with acute exposure but not ending, over time there will be recruitment of additional pathways of immune activation.

In addition to direct activation of innate immune mechanisms, pattern recognition receptors can activate enzymes (MASP-2) of the mannose binding lectin pathway of complement to cause activation of C4 which can in turn lead to activation of C3. Split products of complement activation, C3a and C4a, also called anaphylatoxins, are markers of innate immune activity as well as initiators of additional aspects of innate immune responses, causing degranulation of basophils and mast cells; recruitment of neutrophils and chemokines; stimulation of smooth muscle spasm, likely to include pre-capillary

sphincters; as well as additional activation of MASP2. MASP-2 can auto-activate; providing for ongoing manufacture of C4a even in the absence of exposure. Ongoing elevated levels of C4a are routinely seen in affected patients even though each C4a molecule itself is short-lived.

Control of this seemingly endless cascade of pro-inflammatory responses comes from compensatory release of anti-inflammatory cytokines (especially IL-4, IL-8, IL-10; Th-2 responses) but more importantly, inflammatory responses are under regulatory control from neuropeptides, especially alpha melanocyte stimulating hormone (MSH) and vasoactive intestinal polypeptide (VIP).

Even with adequate stores of MSH and VIP, unopposed inflammatory responses impair further production of MSH and VIP, leading to loss of regulation of inflammatory responses. If the antigenic stimulus that leads to loss of MSH and VIP continues, the one-sided inflammatory responses accelerate without negative feedback control.

Acquisition of deficiency of MSH and VIP results in multiple immune and hormonal abnormalities.

Additional elements seen in illnesses characterized by innate immune activation include abnormalities in coagulation. Endotoxin-associated illnesses, including bacterial sepsis, dominate the literature in this respect, but much of the rheumatologic/immunologic literature focuses discussion on chronic inflammatory response syndromes. Early in the CIRS-WDB literature there was much discussion of hemoptysis as a result of exposure of children in Cleveland to WDB that hosted *Stachybotrys* species, though no evidence was presented to show abnormalities in coagulation in those small cohorts. Coagulation defects have long been suspected since epistaxis (nose bleeds) and hemoptysis (coughing up blood from the lung) are not uncommonly reported by patients.

We can ask if patients with chronic illness acquired after exposure to the interior environments of WDB (called CIRS-WDB for this discussion) have low levels of MSH and VIP. They do; these deficiencies are seen with a prevalence of 95-98% in cases and less than 5% in controls. In those with low levels of MSH and VIP, was there evidence of abnormalities in regulation of C4a, TGF beta and a measure of pro-inflammatory cytokines (MMP-9)? Yes, that evidence was abundantly more common in patients than controls. Was there evidence of coagulation disturbances seen more in cases than in controls? Yes, there were, with significant differences confirmed in cases compared to controls for all elements of von Willebrand's profile.

Finding these abnormalities is considered to be a type of epidemiologic evidence called association. One can think of association logically as a construct of if A exists, therefore B will be found. Presence of an association does not mean A caused B to appear. One must use an epidemiologic study design that enables us to assess risk in order to prove causation. Incidentally, the GAO had a different view on proof of causation. That report (page 7) says that establishing a causal relationship requires three elements: (1) epidemiologic associations (2) experimental evidence that exposure in humans and

animals leads to symptoms and signs of the disease (3) treatment leads to reduction in symptoms and signs of the disease.

If there is an association of abnormalities in innate immune responses seen in cases after the illness is established, can we show acquisition of these abnormalities in patients observed prospectively with exposure to the interior environment of WDB? Yes, treating physicians use a repetitive exposure protocol, one termed ABB`AB, that involves establishment of a data base A, with correction of abnormalities B using medications, and no change in three days off medication without exposure to a known WDB (B`), followed by three days of exposure to the original suspect building (A) with known water damage and microbial amplification (monitored each day for three consecutive days without use of protective medication), and followed by re-treatment with medication, item B. Next to be shown is that baseline A is identical to re-exposure A and that treatment B, absent re-exposure B` and second treatment also equals B. Further, it is now knows that the abnormal innate immune physiology will result in recrudescence of abnormalities in objective lab testing of a defined sequence with enough consistency that the human health index (Sequential Activation of Innate Immune Elements, SAIIE), collated from lab results and symptoms with reacquisition of illness in the three day observation period, correlates precisely with a building index created by the US EPA, called the Environmental Relative Mold index (ERMI).

Finally, these data have been presented in multiple academic presentations and papers to show the illness is indeed treatable as shown by reduction of symptoms and improvement in signs of the illness.

In the terms of the GAO, treating physicians should have more than just human health data on the effects of exposure causing illness and inflammatory changes: animal evidence is needed as well. That evidence is presented.

Please refer to the bibliography that follows for cited references by number. These references were selected from a total of 4441 hits for the key phrase "mold and inflammation" entered on PubMed 6/3/09.

Mycotoxins:

Ref 1. Trichothecene mycotoxins activate inflammatory response in human macrophages.

Activation of inflammasome associated caspase-1 and enhanced LPS-dependent secretion of IL-1β and IL-18 was shown after trichothecene exposure. This exposure also triggered release of caspase-3, which is an effector of apoptosis. The conclusion is that human macrophages sense trichothecene mycotoxins as a danger signal.

Ref 2. Modulation of LPS-induced pro-inflammatory cytokine production by satratoxins and other macrocyclic trichothecenes in the murine macrophage.

Low concentrations of trichothecenes strongly induce expression of TNF α. At higher concentrations, cell viability was impaired. The capacity of trichothecenes to alter cytokine production may play an etiologic role in human illness.

Ref 3. Intranasal exposure to a damp building mold, *Stachybotrys*, induces lung inflammation in mice.

An increase in inflammatory cells is seen in bronchoalveolar lavage fluid (lung) after intranasal instillation. Induction of cytokine and chemokines was seen, but not different from satrotoxin formers versus non-formers. No increase in methacholine reactivity of IgE was seen. Lung inflammation is regulated by induction of pro-inflammatory cytokines and the mediators of this inflammatory response are distinct from satratoxins.

Ref 4. *Stachybotrys chartarum*, trichothecenes mycotoxins and damp building-related illness: new insights into a public health enigma.

Damp building-related illnesses include a myriad of respiratory, immunologic and neurologic symptoms. *Stachybotrys* evokes inflammation. Toxins form protein adducts in the test tube and in people. They cause neurotoxicity in nose and brain in mouse. They cause pulmonary inflammation. Other compounds that evoke inflammation include proteinases, hemolysins, beta-glucans, and spirocyclic drimanes. Studies must include low dose exposure to mixtures of compounds, including mycelial fragments, spores, and co-exposure with other environmental factors.

Ref 5. Detection of airborne *Stachybotrys chartarum* macrocyclic trichothecenes mycotoxins in the indoor environment.

Airborne trichothecenes were found in WDB but not in control buildings.

Ref 9. Strain differences influence murine pulmonary responses to *Stachybotrys chartarum*.

Some mice strains show a markedly increased pulmonary inflammatory response to instilled spores. This inflammatory response was due to innate immune mediators and was not due to allergy. The responses to spores are not the same as those seen to LPS and other fungi. Genetic differences may contribute to the wide range of sensitivity to *Stachybotrys* among humans.

Ref 10. Comparison of inflammatory responses in mouse lungs exposed to atranones A and C from *Stachybotrys chartarum*.

Exposure to atranones leads to immunotoxic, inflammatory and other pathological changes. Atranones are inflammatory and they exhibit different inflammatory potency. These data suggest that exposure to these toxins in WDB could contribute to human inflammatory lung disease.

Ref 17. Chronic intranasal administration of *Aspergillus fumigatus* spores leads to aggravation of airway inflammation and remodeling in asthmatic rats.

The conclusion of this study is that chronic exposure to *A. fumigatus* aggravated airway inflammation and prompted airway remodeling in asthmatic rats.

Ref 81. Effects of *Aspergillus fumigatus* gliotoxin and methylprednisolone on human neutrophils.

Gliotoxins serve to increase neutrophil-mediated inflammation which is likely to play an important role in tissue destruction

Ref 82. *Aspergillus fumigatus* suppresses human cellular immune responses via gliotoxin-mediated apoptosis of monocytes.

Gliotoxin inhibits antigen-presenting cell function and is cytotoxic to monocytes. Production of gliotoxin is an important immunoevasive mechanism that is mediated by direct effects on antigen-presenting cells and T cells.

Ref 93. Mycotoxin production by indoor molds.

Spirocyclic drimanes are made by *Stachybotrys* and can add to inflammatory burden from exposure to WDB.

Proteinases and proteases:

Ref 84. The role of fungal proteinases in pathophysiology of *Stachybotrys chartarum*.

Proteinases from spores contribute significantly to lung inflammation and injury as shown by exceedingly high levels of cytokines in cases compared to controls.

Ref 80. Inflammatory effects of environmental proteases on airway mucosa.

Proteases stimulate a tissue response that is a form of innate immunity directed against organisms.

Endotoxin inhalation:

Ref 33. Moldy environments and toxic pneumonitis.

Exposure to endotoxins and cell wall agents can cause a toxic pneumonitis. Inhalation of endotoxin, molds and fungal cell wall agents were tested. Endotoxins caused an acute toxic pneumonitis.

Ref 34. The inflammatory response in humans after inhalation of bacterial endotoxin: a review.

Inhalation of endotoxin (LPS) causes acute symptoms and lung finding in volunteers. A local and systemic inflammatory response follows inhalation.

Ref 35. Endotoxin in the environment-exposure and effects.

Inhalation causes activation of Toll receptors which results in local release of cytokines, airway inflammation, release of cytokines into the bloodstream, symptoms and disease.

Ref 36. Health effects due to endotoxin inhalation (review).

Toll receptors and IL-1 receptors play a pivotal role in the endotoxin activation cascade. Endotoxin activation cascades cause many adverse effects on human health.

Ref 37. Occupational endotoxin-exposure and possible health effects on humans.

The adverse health effects of endotoxin exposure are known.

Ref 56. Fungal and endotoxin measurements in dust associated with respiratory symptoms in a WDB. (NIOSH study).

Microbial agents in floor dust may be a good surrogate measure for exposure. Both the effects of fungal exposure and endotoxin exposure are necessary.

T-regulatory lymphocytes, IL-17, TGF β-1

Ref 18. Cell mediated immunity to fungi: a reassessment.

Th-17 may play an inflammatory role that accommodates the paradoxical association of chronic inflammatory responses with fungal persistence in the face of ongoing inflammation. Regulatory T-cells are an integral component of immune resistance to fungi and can inhibit aspects of innate immunity (NB: This protection is lost when high levels of TGF beta-1 inhibit T-regulatory function).

Ref 19. Il-17 and therapeutic kynurenines in pathogenic inflammation to fungi.

Innate immune responses are inflammatory. Progressive inflammation (NB: if MSH and/or VIP is low) worsens pathogen inflammation. The Th-17 pathway may favor pathology, and serve to accommodate the seemingly paradoxical association of chronic inflammation with fungal persistence.

Ref. 20. Controlling pathogenic inflammation to fungi.

Dysregulation of inflammation may significantly worsen fungal disease. These new findings provide a molecular connection between the failure to resolve inflammation and the lack of antifungal immune response.

Ref 11. IL-17 producing T cells in lung immunity and inflammation.

Th-17 immunity is systemic and multimorphic.

Ref 12. Th-17 cytokines and their emerging roles in inflammation and autoimmunity.

Accumulating data support the role for Th-17 cells and their cytokines in inflammatory processes and in animal models of autoimmunity and inflammation.

Ref 13. Th-17 cytokines and mucosal immunity.

Th-17 plays a significant role in mediating host mucosal immunity to pulmonary pathogens.

Ref 14. IL-17 and Th-17 cells.

TGF beta-1 induces differentiation of Th-17 cells. This finding opens up a new understanding of T-regulatory cells.

Ref. 62. Th-17 cells: a new fate for differentiating helper T cells.

Initiated by TGF β-1, Th-17 cells play an essential role in host defense against fungi and in pathogenesis of autoimmune disease. Selectively targeting the Th-17 lineage may be beneficial for treatment of inflammatory and autoimmune diseases.

Ref 77. IL-23 and the Th-17 pathway promote inflammation and impair antifungal immune resistance.

Dysregulation of inflammation may significantly worsen fungal diseases. Inflammation is heightened by a Th-17 response and impairs antifungal resistance.

Dectin-1, beta-glucans

Ref 22. Internalization of dectin-1 terminates induction of inflammatory responses.

Dectin-1 is a pattern-recognition receptor that recognizes (1, 3) β-glucans found on fungal cell walls. Dectin-1 induces transcription of innate immune response genes. Dectin-1 receptors are internalized (phagocytosed) after activation, thereby reducing inflammatory responses. Absence of internalization or repeated activation of membrane-bound dectin-1 will enhance inflammatory responses.

Ref 24. The biological role of dectin-1 in immune response.

Dectin-1 is the most important receptor for beta-glucan. Recognition of beta-glucan by dectin-1 provides a pro-inflammatory response.

Ref 25. The role of dectin-1 in antifungal immunity.

Beta-glucans stimulate inflammatory responses by the innate immune system mediated by dectin-1.

Ref 26. Macrophage internalization of fungal beta-glucans is not necessary for initiation of related inflammatory responses.

Beta-glucan induced NF-kappaB translocation is necessary for inflammatory activation.

Ref 38. The induction of inflammation by dectin-1 in vivo is dependent on myeloid cell programming and the progression of phagocytosis.

Dectin-1 is sufficient to drive a potent inflammatory response in a context-dependent manner.

Ref 50. Beta-glucans and dectin-1.

Characterization of beta-glucan sensing, intracellular signaling and induction of cellular response has provided new insights into the role of beta-glucans in immunity and disease.

Ref 63. Branched fungal beta-glucan causes hyper-inflammation and necrosis in phagocyte NADPH oxidase-deficient mice.

Hyper-inflammation induced by beta-glucans was predominantly due to a defect in termination of inflammation.

Ref 67. Macrophage receptors and innate immunity: insights from dectin-1.

Dectin-1 plays a role in the inflammatory response to pulmonary fungal pathogens and is involved with autoimmune and respiratory diseases.

Ref 71. The beta-glucan receptor dectin-1 recognizes specific morphologies of *Aspergillus fumigatus*.

Alveolar macrophages are the first line of innate host defense for clearing inhaled *A. fumigatus* from the lung. Release of pro-inflammatory cytokines was dependent on recognition of *A. fumigatus* by dectin-1.

Toll receptors

Ref 28. The contribution of PARs to inflammation and immunity to fungi.

Activation of Toll receptors by fungi unmasks a divergent role for protease activated receptors (PAR) in inflammation. Inflammation is acquired from the interaction of Toll receptors and PAR.

Ref 29. Recognition of fungal pathogens by Toll-like receptors

Toll receptors are major pattern recognition receptors. Recognition leads to activation of innate immune responses.

Ref 30. Toll-like receptors as key mediators in innate antifungal immunity.

The innate immune system uses Toll receptors to selectively signal after fungal recognition.

Non-Toll receptors

Ref 70. MyD88 signaling contributes to early pulmonary responses to *Aspergillus fumigatus.*

MyD88 dependent pathways mediate acute pulmonary fungal clearance, inflammation and tissue injury within a three day window, demonstrating the importance of mediation of pulmonary inflammatory responses to fungi.

Ref 40. *Aspergillus fumigatus*-induced interleukin-8 synthesis is controlled by non-toll like MyD88 pathway.

Two independent signaling pathways in respiratory epithelium are activated by *A. fumigatus.*

Ref 45. Relevance of airborne fungi and their secondary metabolites for environmental, occupational and indoor hygiene.

Airborne fungal contaminants are gaining importance in view of health hazards caused by spores and secondary metabolites.

Ref 46. Aerobiology-main research field of interest in last 25 years.

Many fungi negatively affect human health and cause immunotoxic diseases.

Ref 47. Airway inflammation in employees involved in cultivating Japanese mushrooms (*bunashimeji*).

The inhalation of spores directly produces toxic inflammatory effects in the airways, independent of the degree of sensitization.

Ref 54. Respiratory inflammatory responses among occupants of a WDB. (NIOSH)

Symptoms are associated with airway inflammation.

General

Ref 79. Control, immunoregulation and expression of innate pulmonary defenses against *Aspergillus fumigatus*.

Innate host defenses include phagocytic cells, cytokines, chemokines, toll-like receptors and antimicrobial peptides.

Ref 31. Complement activation in tear fluid during occupational mold challenge.

C3a is actively produced in tears during mold exposure.

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What Does Human Research Tell Us?

Epidemiologic studies that look at cases versus controls give us a clear indication that following exposure to the interior environment of WDB there is an inflammatory illness with multiple health symptoms from multiple organ systems but those symptoms do not of themselves give us causation of illness. Causation can be obtained following determination prospectively of acquisition of illness (symptoms, laboratory findings and ancillary studies) in a well individual who is exposed or re-exposed to an environment with known water damage and amplification of microbial growth. Prospective exposures of patients with known baseline laboratory findings give treating physicians and observers the ability to assess changes in such laboratory studies with re-exposure.

Understanding that there are concerns from some Indoor Hygienists about ERMI being a reliable assessment tool for building health, the ERMI, and the sum of values obtained from Group 1 + Group 2 that exceeds 30, are two measurements that have been used for nearly four years in human research by physicians practicing in the field. ERMI values have been correlated with (i) capillary hypoperfusion; (ii) safety of re-exposure; and (iii) safety of new occupancy of previously affected patients.

Prior work (Shoemaker R, "SAIIE," IAQA 11/07; Shoemaker R, "When SAIIE meets ERMI," AIHA 6/08) has shown that acquisition of CIRS-WDB is rare if normal levels of alpha melanocyte stimulating hormone (MSH) are found, i.e. the levels exceed 35 pg/ml. For those with CIRS-WDB and MSH deficiency (< 35 ng/ml) and illness, the illness is further stratified by levels of C4a. C4a levels over 20,000 ng/ml at baseline change the magnitude of the inflammatory response seen with additional exposures, making the rise in subsequent levels of adverse inflammatory parameters higher and occurring in shorter periods of time ("sicker, quicker"). In-office data confirm that there is a statistical correlation between the magnitude of rise of C4a with higher ERMI test results. Additional data suggest that if ERMI results exceed negative 1.0, then patients with both MSH deficiency and high C4a will become ill rapidly when exposed to WDB.

For those whose C4a that has never exceeded 20,000, the threshold for future adverse health effects rise to an ERMI of 2.0, understanding that ERMI will only identify mold species and provide an index of building health. It is not quantitative for absolute numbers of organisms. Yet the human health data are clear: for the patients with C4a greater than 2830 ng/ml but less than 20,000 ng/ml, which accounts for the majority of those with illness, any ERMI > 2.0 is associated statistically with acquisition of illness with re-exposure.

Fortunately, the greater number of patients with CIRS-WDB have C4a < 20,000 ng/ml; it is in this group of people that the rate of rise of adverse inflammatory response elements is blunted, with levels not reaching quite as high as levels of those with a response system that is "primed" for a more reactive response.

Said another way, ERMI testing has been correlated with safety for those with prior illness; lower ERMI levels are required for safe occupancy by sicker patients. Much

work is underway in this field, particularly anticipating that ERMI testing will become refined in the future, and that such refinement will be based on the linkage of building indices of health to human indices of health.

When working with patients undergoing repetitive exposure trials, using a standard research design abbreviated as ABB`AB, Shoemaker and others have been able to show that there is a somewhat predictable pattern of change over time of the physiological parameters in response to WDB environments. Following this re-exposure of previously treated patients, one will invariably see from the results of daily blood draws that levels of C4a, a split product of the activation of complement (often called an anaphylatoxin), rise within four hours. Leptin levels will rise in approximately 24-48 hours, reflecting the increased cytokine effects damaging the receptor for leptin in the MSH production pathway. MMP-9 will rise in approximately 48 hours, reflecting the time course for cytokine effect to stimulate gene activation of the MMP-9 production pathway. VEGF levels initially rise rapidly, followed by a significant drop. Clotting factors also change with unprotected re-exposure, with Factor VIII levels falling concomitantly with C4a and then returning to normal after 48-72 hours. Von Willebrand's factor and activity are maintained for 24-48 hours, falling to a low in 72-96 hours. This change is often associated with unexplained bleeding, usually either from the nose or lungs (epistaxis or hemoptysis). While not all patients show all the inflammatory changes described above, *no control* patients show any of the changes over a four day course of daily blood draws.

Furthermore, no changes in any lab parameters performed daily will occur in patients with prior mold illness who are not exposed to WDB.

A second course of therapy with cholestyramine will return the abnormal lab values back towards values seen following initial therapy. These abnormalities in lab testing reflect the sequence of activation of the inflammatory cascade discussed previously.

Causation, then, can be quantified by looking at what percentage of untreated baseline findings is achieved at each of the three days of unprotected (i.e. by not taking cholestyramine) re-exposure to a known WDB. C4a peaks on day1; leptin on day 2; MMP-9 on average between day 2 and day 3; VEGF should rise on day 1 and then fall by day 3. TGF β-1 rises rapidly and remains elevated, as opposed to C4a levels that can decline during additional days of ongoing re-exposure. The abnormalities in clotting studies, together with the unusual finding of so many CIRS-WDB patients showing an acquired von Willebrand's disease coinciding with bleeding from nose and lung, suggest that the inflammatory response syndrome creates a coagulopathy. Specifically, in these cases we see that the low ristocetin associated co-factor and low multimers of vWF antigen, normally seen in 1 in 10,000 patients, are found instead in 3% of CIRS-WDB patients - a **300-fold** magnification of relative risk. In CIRS-WDB patients, this coagulopathy responds rapidly to treatment with DDAVP. Use of this drug enhances polymerization of von Willebrand's multimers by mobilizing multimers that are marginalized in blood vessels (i.e. causing protein fragments to become more sticky), thereby providing a rapid way to stop hemorrhage in CIRS-WDB patients.

Shoemaker's group has used this research design to confirm causation of illness in over 2500 patients. The causation data presented by those papers confirms the value of using baseline parameters to assess causality. Causality, as discussed by Kundi (Kundi M; Institute of Environmental Health, Center for Public Health, Medical University of Vienna, Austria. Causality and the interpretation of epidemiologic evidence Environmental Health Perspectives 2006; 114(7): 969-974), describes the epidemiologic reliability of illness presentation. Key to his discussion is that there is a commonality of findings, both symptoms and lab parameters, seen across groups and across studies. These concepts are embodied in the GAO case definition which requires (i) epidemiologic similarity to other human cases and (ii) findings in the cases that are similar of human and animal research, with (iii) response to therapy. Published case rosters in adults and children meet the criteria described by Kundi and GAO such that even without prospective confirmation of re-acquisition of illness with re-exposure, baseline parameters ensure that treatment will improve clinical outcome. What that means is that treating physicians can develop at the initial office visit an understanding as to whether or not a patient has CIRS-WDB based on preliminary lab results and ancillary testing such as visual contrast sensitivity (VCS). Kundi's paper is a marked upgrade from Bradford Hill of 1965. Kundi provides a framework for discussion of causality – he describes that the consistency of demographics and environmental exposure at baseline combined with the weight of epidemiologic evidence is adequate to infer causality.

Treating physicians then have a duty to record baseline data, including symptoms and laboratory parameters, recognizing that there is no requirement for independent practitioners to alter their tailored therapy to suit a causality paradigm.

Treatment

Given the lack of consensus until the GAO report of 2008 that CIRS-WDB was an inflammatory and an immunologic condition as well as a neurotoxic condition, it is not surprising that treatment protocols for the illness have not been well-discussed in peer reviewed literature before 2009. We note that controlled studies that present results of salutary changes in health from a given intervention that result in measurable changes of objective parameters are under-represented in the world's literature. Recent approaches to improving health of *people* exposed to WDB have focused on *building* factors, for example:

1. **Improving ventilation**. Seppanen O, Fisk W. Indoor Air 2004; Suppl 7: 102-118. Summary of human responses to ventilation.

2. **Architectural changes**. Small B. Toxicol Ind Health 2009; 25 (9-10): 731-5. Creating healthier buildings.

Renovation.

1. Buchanan I, Mendell M, Mirer A, Apte M. Indoor Air 2008; 18(2): 144-55. Air filter materials, outdoor ozone and building related symptoms in the BASE study.
2 Hiipakka D, Buffington J. Appl Occup Environ Health 2000 15(8): 635-43. Resolution of sick building syndrome in a high-security facility.
1. Huttunen K, Rintala M, et al. Environ Res 2008; 107(3): 291-8. Indoor air particles and bioaerosols before and after renovation of moisture-damaged buildings: the effect on biological activity and microbial flora.
2. Lahtinen M, Salonen H, et al. Am J Ind Med 2009; 52(5): 438-45. Renovation of a "sick building": the challenges of obtaining the confidence of occupants.

Each of these recent studies ignores the repeatedly observed findings that inflammatory changes acquired following exposure to WDB *will not abate* with simple removal from exposure, remediation of the affected structure or prevention of future exposures in those with genetic susceptibility to the inflammatory illness. Said another way, even if you make a bad building safe, the inflammatory injury caused by the WDB persists even though the prior microbial growth stops and the inflammagens are removed.

Any discussion of treatment must be based on a well-defined diagnosis, made using a rigorous process of differential diagnosis. To establish the presence of CIRS-WDB, there must have been exposure to the interior environment of a WDB. Microbial amplification (bacteria, fungi, actinomycetes) begins in approximately 48 hours after water intrusion. If the intrusion is ongoing such as from an internal plumbing leak, the organisms than can be expected to be present include those commonly found in water-saturated conditions, including *Stachybotrys* and *Chaetomium*, for example. Episodic water intrusion, such as from flood events and roofs leaks, will be expected to show a greater predominance of *Aspergillus* and *Penicillium* species. Microbial testing, if performed, will be expected to

reflect the ecological disturbances found that made the building a WDB. In the absence of sophisticated PCR testing or ERMI, confirmation of the building being a WDB can be obtained by documentation of visible mold and or musty smells. Understanding that the sensitivity to smells (olfactory acuity) varies from person to person, absence of musty smells according to a given observer is not adequate to rule out microbial growth.

Any water intrusion event must be dried out and cleaned up within 48 hours or predictable microbial amplification will begin indoors. Common errors in remediation, such as ignoring carpets, OSB sub-floors or wall cavities as reservoirs of microbial growth after 48 hours of water intrusion will predictably lead to an increased risk of illness to patients occupying that indoor space.

Once there is evidence that a building is truly water-damaged, then there must be epidemiologic similarity of the findings from a given patient to those findings identified in published cohorts of CIRS-WDB patients, including symptoms and laboratory data. The illness is multisystem and multisymptom. The diagnosis of CIRS-WDB can be excluded if such diversity of symptoms is not found. Similarly, absence of laboratory confirmation of the inflammatory nature of the illness and demonstrating that there is no reduction of regulatory neuropeptides or associated dysregulation of hormone physiology rules out the diagnosis. Proper relevant testing must be done to conclude that the abnormalities are not present. Absence of testing does not suffice to say that the test results are absent.

Absence of proof is not proof of absence.

Adjuncts to diagnostic accuracy include finding deficits in visual contrast sensitivity (VCS), a neurotoxicologic test that is well-represented in published literature and is used by Federal agencies including EPA, NIOSH and the CDC.

Finding presence of particular HLA DR haplotypes adds weight to the differential diagnosis process but absence of such HLA DR does not rule out the diagnosis.

It is clear at this time that no single test can diagnose CIRS-WDB illness, though genomic assays are promising in this regard for the future. It is the **constellation of laboratory findings that document the CIRS**, with each test simply reflecting the given part of the inflammatory process itself. The many particular tests have been discussed previously but the treating physician must account for neuropeptide regulation of inflammation (MSH, VIP); inflammatory processes out of control (TGF beta-1, C4a and MMP9); genetic susceptibility (HLA DR); hormonal dysregulation (ADH/osmolality, ACTH/cortisol, androgens); hypoxia inducible factor regulated compounds (VEGF, erythropoietin, TGF beta-1); autoimmunity (TGF beta-1, AGA, ACLA, ANA, ANCA, actin) and the cellular basis of immune regulation (TH17 immunity, TGF beta-1).

Further, treating physicians must remain aware of the likelihood of co-occurring illnesses in CIRS-WDB patients compared to non-cases. For example we commonly find restrictive lung disease and not true obstructive lung disease; decreased contractility of

gastric smooth muscle causing gastroparesis; cholecystopathy causing bile acid reflux; encephalopathy causing cognitive disorders and atypical headaches; mucosal inflammation causing malabsorption and bowel disorders; connective tissue inflammation producing rheumatologic and chronic pain disorders and increased prevalence of autoimmune disorders, among others. Finally, unusual neurologic conditions (tremors, atypical seizures, neuropathic pain) are quite commonly seen in patients with elevated TGF β-1 levels. Each of these conditions does not diagnose CIRS-WDB but is quite commonly found among our affected patients.

The lessons from the treating physicians, those who know the illness and the patients best, are that CIRS-WDB must have specific targeted therapies to return sickened patients to a modicum of health. Shoemaker's data on over 6000 treated patients (with more than 2000 cases and 450 controls published) shows that a significant percentage of patients will have persistently lowered levels of the regulatory neuropeptides alpha melanocyte stimulating hormone (MSH; less than 35 pg/ml) or vasoactive intestinal polypeptide (VIP; less than 23 pg/ml) despite all therapies. Adequate control of inflammatory responses to exposure to the interior environments of WDB demands adequate neuropeptide regulation of inflammation. CIRS-WDB patients who lose this regulation will continue to have increased susceptibility to relapse with re-exposure to the interior environment of WDB. Current research into use of replacement VIP has resulted in marked reduction of the "sicker, quicker" phenomenon and has restored health to those for whom other treatments had been ineffective. While this early research is promising, much more work needs to be completed in this emerging field.

Without adequate neuropeptide regulation of inflammation, a predictable series of events follows. Hormonal regulation of androgens, antidiuretic hormone and ACTH are each compromised with an incidence of over 50%. Gastrointestinal functions deteriorate. Abnormalities in gene activation under the control of hypoxia inducible factor (HIF) occur, particularly when low levels of VEGF are present. TGF β-1, once unleashed, is responsible for (i) restrictive lung disease; (ii) autoimmunity; and (iii) neurologic abnormalities. We now know that correction of VIP deficiency yields beneficial effects, provided that the patient is not further exposed to a WDB.

Successful correction of the series of abnormalities of the systemic inflammatory and innate immune responses is mandatory before successful treatment of the illness can be accomplished. Correction of symptoms; neurotoxicologic deficits (i.e. VCS); inflammatory markers (C3a, C4a, MMP9, TGF β-1); presence of commensal biofilm formers (i.e. multiply antibiotic resistant coagulase negative staphylococci); presence of autoimmune findings and VIP deficiency must take place to see resolution of illness. Given that not all patients have all such abnormalities, the patient must be assessed at baseline for these potential problems. Identification of all known physiological abnormalities provides both (i) substantiation of the chronic inflammatory basis for the illness and (ii) a platform from which to direct therapy.

Without documentation of resolution of the inflammatory abnormalities seen universally in CIRS-WDB, the treating physician is left with monitoring subjective changes in

symptoms as markers for improved health. Symptoms are a reasonable indicator of health, taking into account that there is significant variation of symptom expression among individuals. Yet symptom recording itself is subject to a variety of potential biasing elements including use of a checklist, illness stage at the time of symptom recording, potential for presence of undiagnosed confounding factors, and attitudinal variation on the part of the patient and health professional recording the symptoms. Two large studies of patients with treatment-confirmed CIRS-WDB provide a roster of symptom prevalence in populations (see treatment protocol references below).

In order to properly assess the indications for therapy and response to treatment, we must have objective parameters that support and validate symptoms.

Validation of exposure to a WDB and the subsequent responses to treatment is supported by human studies in which patients provided informed consent for re-exposure to the interior environment of WDB without use of protective medication.

We know that management of such complex illness is necessarily complex. We have recorded treatment protocols from several physicians who provided algorithms derived from their extensive work experience. Each of these physicians has data to support use of their protocol. We are not suggesting that there is any single approach to treating CIRS-WDB, much as there is not just one strategy for management of hypertension or diabetes, for example.

As further research unveils newer modalities that can be shown to be safe and effective, the treatment protocols listed will necessarily be revised. Absence of inclusion of a given protocol does not suggest lack of efficacy; rather that the protocol was either not previously published in a peer-reviewed publication or submitted for this position statement. We are aware that there are a number of treating physicians who describe successful treatment of CIRS-WDB patients using additional therapeutic modalities described as individualized, with responses to treatment described anecdotally. We encourage all clinicians involved with treatment of CIRS-WDB to adopt rigorous and replicable diagnostic and response parameters in order to facilitate comparisons between various treatment modalities. Most importantly, obtaining pre- and post-treatment laboratory data objectively confirms therapeutic benefit and substantiates subjective clinical reporting.

Treatment Protocols:

A. Ritchie C. Shoemaker MD Pocomoke, Md

Based on recorded findings from 13 years of work in the field covering over 6000 patients the following therapies, taken strictly in order, are known to provide benefit:

1. Removal from exposure
2. Use of orally administered cholestyramine or Welchol, anion binding agents, taken four times a day in therapeutic doses until VCS scores are normalized.
3. Eradication of commensal, biofilm-forming, multiply antibiotic resistant coagulase negative staphylococci. These organisms are usually methicillin resistant.
4. Correction of antigliadin antibody positivity by three months (at least) of gluten avoidance, provided that celiac disease is ruled out by negative TTG-IgA.
5. Normalization of MMP9.
6. Correction of ADH/osmolality dysregulation.
7. Correction of androgen deficiency/aromatase upregulation.
8. Correction of C3a followed by
9. Correction of C4a.
10. Correction of elevated TGF β-1.
11. Replacement of VIP in those deficient in VIP.

Particular attention needs to be made to correction of elevated lactate seen on magnetic resonance spectroscopy and correction of abnormal ratio of glutamate to glutamine also seen on MR spectroscopy if such abnormalities persist beyond correction of elevated C4a.

B. Scott McMahon MD, Roswell, New Mexico

Based on one year in the field covering 100 patients

1. Removal from exposure.
2. Use of orally administered cholestyramine or Welchol, anion binding agents, taken four times a day in therapeutic doses until VCS scores are normalized.
3. Eradication of commensal, biofilm-forming, multiply antibiotic resistant coagulase negative staphylococci. These organisms are usually methicillin resistant.
4. Correction of antigliadin antibody positivity by three months (at least) of gluten avoidance, provided that celiac disease is ruled out by negative TTG-IgA.
5. Normalization of MMP9.
6. Correction of ADH/osmolality dysregulation.
7. Correction of androgen deficiency/aromatase upregulation.
8. Correction of C3a followed by
9. Correction of C4a.
10. Correction of elevated TGF β-1.

C. Laura Mark MD, Williamsburg, Virginia

Based on one year in the field covering 25 patients evaluated with blood work from a cohort of 100 affected and exposed patients identified from a base of 500 psychiatric patients. No treatment provided but differential diagnosis parameters tested and recorded in a database. Focus of therapy initiated was to educate patient and family; referring physician(s); and involved staff regarding exposure and treatment considerations as below.

1. Removal from exposure.
2. Use of orally administered cholestyramine or Welchol, anion binding agents, taken four times a day in therapeutic doses until VCS scores are normalized.
3. Eradication of commensal, biofilm-forming, multiply antibiotic resistant coagulase negative staphylococci. These organisms are usually methicillin resistant.
4. Correction of antigliadin antibody positivity by three months (at least) of gluten avoidance, provided that celiac disease is ruled out by negative TTG-IgA.
5. Normalization of MMP9.
6. Correction of ADH/osmolality dysregulation.
7. Correction of androgen deficiency/aromatase upregulation.
8. Correction of C3a followed by
9. Correction of C4a.

References on therapy from Dr. Shoemaker's group:

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Biographies and Conflict of Interest

Ritchie C. Shoemaker M.D. is a practicing physician in Pocomoke, Maryland where he has practiced since 1980. He received his M.D. degree from Duke University in 1977. He attended Family Practice residency in Williamsport, Pennsylvania from 1977-1980. He was Board Certified in Family Practice from 1980 to 2006. His practice is now devoted exclusively to diagnosis and treatment of those with chronic fatiguing illnesses. He is involved in studies with multiple collaborating researchers.

He reports receiving income from testimony in cases involving mold litigation. He has interest in a website www.chronicneurotoxins.com that provided on-line VCS testing for patients.

Dr. Shoemaker is the author of Mold Warriors (2005) and Surviving Mold (2010).

He is Medical Director (unsalaried) of Center for Research on Biotoxin Associated Illnesses, a 501-c-(3) non-profit group that raises funds to perform medical research and funds indigent patients needing on-site treatment for CIRS-WDB.

Laura Mark M.D. is a practicing psychiatrist in Williamsburg, Virginia. She received her M.D. degree from Georgetown University in 1981. She attended residency in Maine Medical Center from 1981 to1985. She is Board Certified in Psychiatry.

She reports no conflicts of interest.

Scott McMahon M.D. is a practicing pediatrician in Roswell, New Mexico where he has practiced for 18 years. Dr. McMahon received his M.D. at Creighton University and completed his Pediatric residency at Duke University. He currently sees children and mold patients through La Casa de Buena Salud, a federally qualified health clinic satellite in Roswell.

He reports receiving income from testimony in one case involving mold litigation.

Jack D. Thrasher Ph.D. received his Ph.D. in Cell Biology from UCLA in 1964. He has extensive experience as a toxicologist and educator. He specializes in Immunotoxicology.

He reports receiving income from testimony as an expert witness for both defendants and plaintiffs in toxic tort cases, including mold litigation.

Carl Grimes HHS CIEC graduated from the University of Denver in 1972. He is President of Healthy Habitats LLC and the Indoor Air Quality Association. He is a consultant living in Denver, Colorado.

He reports receiving income for work providing litigation support.