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Indoor Environment Resulting From Water Intrusion, Part I

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The potential harmful effects of exposure to fungi (molds) in inhabited buildings were recognized and documented in early Biblical times. In the Old Testament (King James Version, Oxford), 1888 Edition, Chapter XIV: (Verses 34 thru 47). Leviticus put forth a detailed protocol for the remediation of mold contaminated structures, including the destruction of dwellings and personal belongings if remediation failed. Presently, it is recognized that water intrusion into buildings leads to amplification of fungi and bacteria (1-11). The Centers for Disease Control (CDC) and Environmental Protection Agency (EPA) recommend re-mediation when water-damage and fungus growth are evident in a home or office building. What has changed since the pronouncement of Leviticus? Presently, we have greater knowledge regarding the types of microorganisms, their toxic by-products, the potentially toxic environment created by water-intrusion and the associated risks of adverse health effects upon occupants of these structures.

Occupants of water-damaged buildings develop multiple organ symptoms and have adverse affects to the upper and lower respiratory tract, central and peripheral nervous system, skin, gastrointestinal tract, kidneys and urinary tract connective tissue, and the musculoskeletal system. Peer reviewed literature discusses adverse health effects with confidence intervals of 95 % or higher. The human response or immune reaction usually falls into one or more of the following four general types of immune reactions:

• a Type I reaction otherwise commonly referred to as an IgE or allergy mediated response;
• a Type II reaction otherwise referred to as a cytotoxic reaction. Target molecules on the cell surface and initiate processes leading to the death of that specific cell. (eg. Hemolytic anemia);
• a Type III reaction otherwise called “immune complex” reactions. Protective antibodies attach to an antigen and initiate an inflammatory reaction or response (eg. Glomerulonephritis);
• or a Type IV reaction which involves the bio-mechanisms of cell mediated immunity or the bodies immunological competency to respond appropriately to foreign tissues, certain infectious agents, chemicals and cancerous cells. (12)

Human illness caused by fungi and bacteria can result in one or all of the following:
• mycotic infections (mycoses) or infectious bio-mechanisms
• fungal rhino-sinusitis;
• IgE mediated sensitivity and asthma;
• hypersensitivity and related pulmonary inflammatory disease;
• cytoxicity;
• immune suppression/modulation;
• autoimmune disorders mitochondrial toxicity;
• carcinogenicity;
• renal toxicity;
• neurotoxicity;
• and adducts to nuclear and mitochondrial DNA causing mutations.

Finally, in the infectious state, fungi and bacteria secrete extracellular digestive enzymes, hemolysins and toxins that cause tissue destruction, angio-invasion, thrombosis, pulmonary bleeding, infarction and other manifestations of the infectious state (for review see references 13, 49, 52).

The purpose of this article is to succinctly review the state of art with respect to indoor air contaminants resulting from water intrusion (damage) and the associated health effects caused by human exposure to this mixture. Indoor environmental biocontaminants discussed in the peer reviewed literature can be grouped into the following categories:

(1) Microbes (fungi and bacteria);
(2) Particulates;
(3) Mycotoxins;
(4) Volatile Organic Compounds;
(5) Extracellular digestive enzymes and Hemolysins;
(6) Extracellular polysaccharides; and
(7) Endotoxins.

By categorizing the indoor environment contaminants based on their general properties, we can simplify discussion of the bio-contaminants and their role in compromising the indoor environment.

1. **Microbes**: The microbes consist of (a) fungi (molds) and (b) bacteria.

a. **Fungi (molds)**: Once water intrusion occurs the fungi begin to grow within 48 hours. They enlarge into more massive growths called colonies. The colonies have different colors, depending upon the specific fungus, e.g. amber, orange, gray, green and black (and sometimes the growth medium). Wherever water is found in sufficient concentration they grow on surfaces as well as being hidden in the carpet, behind wall paper, inside interior and exterior walls, attic, sub floors, etc. The most common fungi identified growing on various substrates (particle board, dry wall, carpeting, etc.) consist of the following genera: *Cladosporium, Aspergillus, Penicillium, Chaetomium, Epicoccum, Alternaria, Trichoderma* and *Stachybotrys*. Certain species of these genera amplify indoors vs outdoors: *Aspergillus* species commonly identified include *flavus, versicolor, sydowii, niger*, and *fumigatus* and the species of *Penicillium* include *chrysogenum, brevicompactum, citrinum,* and *decumbens*. *Stachybotrys chartarum* when present predominantly consists of two chemotypes. The most dangerous chemotype identified is the one that produces trichothecene mycotoxins, while the other chemotype releases atranones. Both strains cause inflammation and have proved to be cytotoxic in mouse lungs (14). The indoor fungal profile is constant when compared to outdoor fungi with respect the presence of species of *Penicillium* and *Aspergillus* being dominant (15, 16). In addition *Stachybotrys chartarum* is readily cultured from indoor versus outdoor environments.

Fungi grow on various building materials based upon water content. Fungi gather nutrients from dead organic material (wood, dry wall, paint, paper glues, etc.) by secreting digestive enzymes into the matrix upon which they are growing. The digestive enzymes break down the organic matter for absorption. The moisture content of the material upon which fungi grow is critical and is called water activity, aw. The aw is the ratio of the vapor pressure exerted by water in the material to the vapor pressure of pure water at the same temperature and pressure. Thus, the fungi are divided into **primary, secondary and tertiary colonizers** depending

upon the aw (5, 17). The partial list of fungi that grow at various water activity ratios given below is helpful for initial basic understanding.

primary colonizers (aw <0.85): *Alternaria citri, Aspergillus (Eurotium) amstelodomi, Aspergillus candidus, glaucus, niger, penicilloides, repens, restrictus, versicolor; Paedilomyces varlotti; Penicillium aurantiogriseum, brevicompactum, chrysogenum, commune, expansum, griseoflavum, commune, expansum, greiseofulvum; Wallemia sebi;*
secondary colonizers (aw = 85-90): *Cladosporium cladosporoides, herbarum, sphaerosperumum; Mucor circinelloides; Rhizopus oryzae;*
tertiary colonizers (aw = >90): *Alternaria alternata; Aspergillus fumigatus; Epicoccum species.; Exophiala species; Fusarium moniforme; Mucor plumbeus; Phoma herbarum; Phiaosphora species; Trichoderma species; Stachybotrys chartarum; Ulocladium consortiale; Rhodotorula species; Sporobolomyces species; Actinomycetes (Actinobacteria).*

b. Bacteria: These consist of gram positive and gram negative organisms. Gram is a stain that penetrates bacteria and helps differentiate and classify the bacteria into positive and negative organisms.

Examples of gram positive bacteria present in water damaged buildings are the *Actinomycetes (Actinobacteria)*. This includes several species of *Streptomyces, Mycobacterium* as well as *Nocardia*. These are filamentous bacteria and produce secondary byproducts. *Streptomyces californicus* produces spores that are approximately 1 micron in size that penetrate deep into alveolar spaces of the lungs. *In vitro* and *in vivo* studies have shown the spores of *Streptomyces* species contain toxins that function as human proinflammatory mediators that can affect the lungs. In addition, the byproducts of S. *californicus* act synergistically *in vitro* with mycotoxins, increasing the toxicity of both byproducts. (9, 18-20). The *Nocardiopsis* strains isolated from indoor water damaged environments are also toxigenic (21). Finally, the CDC recognizes that *Mycobacterium avium, terrae and immunogenum* have been implicated in out breaks if hypersensitivity pneumonia (22). Asthma in adults and children as well as a cluster of inflammatory rheumatic conditions has been attributed to fungus and bacterial contamination in water-damaged buildings and homes (23-27). Other Actinobacteria in the indoor environment are *Micrococcus* species. Examples of other Gram positive bacteria are species of *Atrhrobacter, Bacillus, Cellumonas, Gordona, and Paeniibacillus*(1).

Gram negative bacteria have also been identified in water-damaged buildings. The potential danger of this group of bacteria is the production of endotoxins (lipopolysaccharides) and potential infections, particularly species of *Pseudomonas*. Other gram negative bacteria are species of *Agrobacterium, Caulobacter, Stenophomonas* and *Chryseomonas* (1, 19-20).

2. **Particulates:** Colonies of fungi and bacteria shed particulates into the indoor air, which range from <0.2 to 9 microns. The particulates can be subdivided into two fractions: (a) Large particulates and (b) fine particulates. The large particulate fraction consists of fungus spores and fragments of fungal mycelia (hyphae fragments) hyphae which range from 2 to approximately 9 microns. They are identified by collecting several liters of room air on a membrane filter with a pore size of 2 microns. The large particulates contain mycotoxins, antigenic material, enzymes, hemolysins and other potentially toxic materials. These particulates have been the subject of numerous studies demonstrating their affects on the respiratory tract of mice and rats. Particular attention has been paid to the spores

and hyphae fragments of S. *chartarum* because of its isolation from the lung of a child and its potential role in pulmonary bleeding and hemosiderosis (28-31).

The fine particulate (less than 2 microns) matter consists of material released by both fungus and bacterial growth (5-6). This fraction is shed into the indoor air at activity levels that occur in the frequency range of 1 -20 hertz. Moreover, these frequencies are representative of normal human activity levels, e.g. talking, walking, television, radio, etc. The amount of shed fine particulates is 320 times greater than the large particulate fraction released from fungus colonies. Furthermore, this fraction also contains the byproducts (mycotoxins, endotoxins, antigens, hemolysin, etc.) produced by fungi and bacteria. The aerodynamics of the fine particulates allows them to be inhaled deeply into the microscopic alveolar spaces of the otherwise unreachable areas of the lungs. Then simple diffusion takes place between the particulates and the blood, which permits the entrance of mycotoxins and other toxins into the systemic circulation (5, 6). This has been demonstrated in a series of studies with respect to the fungus *Stachybotrys chartarum*. First a laboratory bench study demonstrated that the trichothecenes from S. *chartarum* are present in the fine particulates (32). Three studies showed that the trichothecene mycotoxins from this organism are present in the fine particulates filtered from the indoor air of homes infested with S. *chartarum* (33-37).

Additional research points towards the fine particulates as a source of toxins. S. *chartarum* does not readily shed its spores into the indoor air and arguments have been made that the concentration of these spores cannot reach critical levels to be toxic to humans. This fungus produces several different trichothecene mycotoxins as well as a hemolytic protein, Stachylysin. Trichothecene mycotoxins have been identified in the blood and urine of symptomatic individuals occupying buildings where this organism was detected (34-36). Stachylysin was shown to be present in the blood of symptomatic individuals at an average concentration of 371nanograms/milliliter (38). This occurred in spite of the fact that these authors could not account for the results of their study based upon indoor air spore counts. More recently it has been demonstrated that exposure of humans and animals to Satratoxin G (a trichothecene) leads to the production of antibodies against Satratoxin G albumin adducts (39), confirming earlier research demonstrating the presence of antibodies to the mycotoxins in symptomatic exposed humans (40).

3. **Mycotoxins:** Mycotoxins are secondary metabolites produced by some fungi (for further information see 13, 17, 41). Most of these mycotoxins enhance the fitness of the fungi in nature. However, they can also cause illness in humans and animals at low concentrations. The illness is referred to as mycotoxicosis. Mycotoxicoses include aflatoxicosis, ochratoxicosis, trichothecene toxicosis, citreviridin toxicosis, fumonisin toxicosis, and gliotoxin toxicosis. In addition, mycotoxins can modulate the immune system as well as inhibit protein, RNA and DNA synthesis. Moreover, they can adduct (bind) to cellular constituents, e.g. DNA, proteins, mitochondria, and cell receptors altering their function. Mycotoxin producing fungi of animal and human health concerns are listed in Table 1. For a copy of Table 1, **Click Here**.

The neurotoxic mycotoxins include ergot alkaloids, trichothecenes, citreviridin, patulin, fumonisins and tremorgens. The neurotoxicity of the tremorgens has been investigated in laboratory animals. They have been shown to affect the brainstem, stellate ganglion, and Purkinjee cells of the cerebellum. Mycotoxins can affect neuroreceptor sites (e.g. gamma-aminobutyric acids (GABA and inositol 1, 4, 5 trisphosphate receptor, inhibit acetylcoholinesterase, release excitatory

neurotransmitters (e.g., glutamate aspartate, GABA, serotonin) and block biosynthesis of complex sphingolipids through the inhibition of ceramide synthetase (for review see 13, 71). Finally, Zearalenone and zearalenol are potent estrogenic compounds already associated or correlated with increased incidence rates of infertility, abortion and uterine prolapse in livestock.

4. **Volatile Organic Compounds.** Fungi and bacteria release volatile organic compounds (VOCS) into the indoor air. It is well accepted that VOCS are irritating to the mucous membranes of the eyes, nose, throat and lungs in settings of sick building syndrome. Thus, the VOCS emitted by microbial growth and water intrusion are an additional factor for consideration in the evaluation process of the indoor environment as contamination is investigated - bearing in mind emissions also occur from newly furnished environments. The VOCS emitted by fungi and bacteria include 2-ethyl-1-1hexanol, 1-butanol, 3-methyl-1-butanol, 2-methyl-1-propanol, terpineol, 2-heptanone, 1-octen-3-ol, dimethyl disulfide, 2-hexanone, 3-octanone, 2 pentylfuran, Aldehydes, ammonia, and various amine compounds (42-46, 71).

5. **Extracellular Enzymes, Siderophores and Hemolysins and Pulmonary Hemorrhage.** Fungi secrete a variety of enzymes that allow them to digest the substrate upon which they grow into their surroundings. This occurs on building materials and also occurs in human tissue in infectious states. The enzymes include lipases, proteinases, metalloproteinases, fibrinolytic enzymes, galactosidases, siderophores, and hemolysins among others. In the human body these secreted enzymes can also become allergens, resulting in an IgE allergic response as well as non IgE mediated lung disease. In addition, they are inflammatory leading to lung disease and the release of proinflammatory cytokines (47-55).

An outbreak of infant pulmonary hemosiderosis was reported in Cleveland and was initially associated with the presence of *Stachybotrys chartarum* (53, 54). Eventually a hemolysin (Stachylysin) and a siderophore were quantified from strains of Stachybotrys isolated from the infants' homes and from a lung of a child with pulmonary hemorrhage (53, 54). In addition, this same investigative team (54) analyzed the dust from these homes for fungi and the production of hemolysins. Eleven species of *Aspergillus*, ten species of *Penicillium*, two species of *Ulocladium, Paecilomyces variotil, Memnoniella echinata, Scopulariopsis bervicaulis, Trichoderma longibrachiatum* and *viride* and *Stachybotrys chartarum* were demonstrated to cause hemolysis of sheep's blood agar. Hemolysins were more commonly produced by the fungi from homes with pulmonary hemorrhage (42%) than from reference homes (10%). These observations are significant in that they demonstrate the complexity of the indoor environment resulting from fungal contamination.

The Cleveland cases were criticized because it was felt by the CDC that sufficient care was not taken in the initial evaluation to implicate *Stachybotrys chartarum*. In retrospect, it would have been more judicious to include all that is currently known about these indoor environments. This would include the following: 1) Fine particulates that contain mycotoxins and other fungal byproducts (5, 6, 32-37). These occur up to 320 greater concentration than mold spores; 2) The fact that stachylysin and trichothecenes are present in body fluids when such findings cannot be attributed to the ambient spore count; 3) Several species of several different fungi do produce hemolysins and siderophores that could also account for the pulmonary bleeding; and 4) Both gram negative and gram positive bacteria co-exist with the fungi and add their toxic byproducts to the mixture. It must be

remembered that the mold spores and hyphae fragment are primarily removed from inhaled air in the nasal cavity. The much smaller fine particulates enter the alveoli where simple diffusion occurs and their contents are able to directly affect surrounding tissue elements and also get into the systemic circulation.

6. **Extracellular polysaccharides (EPS).** The cell wall of fungi is a complex structure mainly composed of polysaccharides and from where the majority of antigens, proteins, polypeptide-polysaccharide (EPS) of the fungus are secreted into the surrounding environment. Some of the components of the wall are directly associated with the colonization of the host tissue and others with damage to the same tissues. For example, the genes and their products with respect to *Aspergillus fumigatus* and other fungi have been reviewed above (45-55).

Two EPS compounds of medical importance are 1, 3 beta-D-glucans (glucans) and galactomannans. Both are diagnostic markers for fungal infections, particularly, *Aspergillus species*, systemic *Candidiasis*, and exposure to several other genera of fungi (49, 52, 55-58). The glucans have been demonstrated in the indoor air and dust and their presence is related to fungal growth as well as intrusion of outdoor sources into homes or buildings (60, 61). The glucans cause airway inflammation and they have been identified in brochoalveolar lavage fluid from individuals with acute eosinophilic pneumonia (62, 63). In addition, they have been reported to potentiate airway allergic conditions by down regulating IgE and promoting airway eosinophil infiltration against inhaled antigens (64, 65). In children this effect is seen as an increased variability in peak expiratory flow probably through nonallergic mechanisms in atopic asthmatic children (65).

Endotoxins. Endotoxins are lipopolysaccharide complexes that are part of the outer cell wall of bacteria. They are associated with gram-negative bacteria, usually pathogens such as *E. coli, Salmonella, Shigella,* and *Pseudomonas*, etc. The endotoxins are maintained within the outer cell wall until autolysis of the bacteria occurs, releasing them into the surrounding environment. They are not only pyrogenic (fever production) but also are antigenic as well as causing inflammation through the activation of the complement system and the TLR4-signaling pathway. They are present in the indoor environment of normal water-damaged homes and buildings (5, 60, 68, 69). In animal models and in humans endotoxins have a synergistic role with the TLR4- signaling pathway leading to increase airway inflammation (5, 26, 65-69).

Summary, Conclusions and Human Health

We have attempted to summarize the complexity of the indoor environment resulting from the presence of fungi and bacteria in water-damaged homes and buildings. This environment is complex containing spores of fungi and some bacteria *(Streptomyces californicus)*, growing and dead fungi and bacteria, fungal and bacterial VOCS, large and fine particulates, mycotoxins, extracellular digestive proteins, hemolysins, and extracellular polysaccharides (EPS) and endotoxins. To isolate a single fraction of this environment, e.g. spore counts, and attempt to indicate from this fraction that adverse health effects upon occupants could not occur is unscientific and irresponsible. Consequently, a California Court recognized these facts under the Kelly-Frye ruling and disallowed defense testimony regarding this issue. The Honorable Michael P. Kenny, Superior Court of California, County of Sacramento, Case # 02AS04291, found that mathematical extrapolations from a rodent study to determine human illness from exposure to molds and mycotoxins is not acceptable science. Judge Kenny ruled …"with regard to Dr. Robbins relying upon her literature review and then jumping to

animal studies and then jumping to modeling conclusions, my ruling is she will not be allowed to present that. There is not a generally accepted view of that particular approach in the scientific community and so therefore it's inappropriate to present that to the jury." Thus, the modeling studies done by GlobalTox were not allowed in the Honorable Kenny's Court.

The court recognized the IOM position in matters regarding modeling from animal data and the Robbins data did not fit this position. The position clearly states *"Risk can be extrapolated from animal studies to human health effects only if chronic animal exposures have produced sufficient information to establish no-observed-adverse-effect levels (NOAELs) and lowest –observed-adverse-effect-levels (LOAELs). Extrapolation of risk exposure from animal experiments must always take into account species differences between animals and humans, sensitivities of vulnerable human populations and gaps in animal data." "Except for a few studies on cancer, toxicologic studies of mycotoxins are acute or short-term studies that use high exposure concentrations to reveal immediate effects in small populations of animals. Chronic studies that use lower exposure concentrations and approximate human exposure more closely have not been done except for a small number of cancer studies." "The results of animal studies cannot be used by themselves to draw conclusions about human health effects."* More will be written on this matter as a follow up article in The Column.

In another case, an Appellate Court decision allowed the testimony of Ritchie Shoemaker, M.D., on behalf of the plaintiff in *Montgomery Mutual Insurance Company v. Josephine Chesson*, et al, No. 1270, September, 2005. The ruling states "Trial court did not err or abuse its discretion in concluding that the methodologies employed by Appellees' expert witness, Dr. Shoemaker, in his determination regarding causation due to exposure to mold were not new or novel scientific techniques requiring application of the Frye-Reed test."

What are the health effects in humans resulting from exposure to indoor environments resulting from water intrusion and microbial growth? There are three monographs and a reviewed research papers.

The first monograph was published by the Institute of Medicine, "Damp Indoor Spaces and Health." (69). The committee met on March 26, June 17 and October 8, 2002. The literature review was predominantly from 2002 and earlier with a few papers published in early 2003. Essentially, the IOM Committee missed the scientific and medical literature published after early 2003. Nonetheless, the committee did conclude on page 212, Tables 5-12 and 5-13, that sufficient evidence did exist to demonstrate upper respiratory tract symptoms, cough wheeze, asthma and hypersensitivity pneumonitis are associated with damp indoor spaces. What the tables also show is that there is "inadequate or insufficient evidence to determine whether an association exists" for several other symptoms and/or health problems. Thus, the Committee did not exclude such health problems as asthma, COPD, lower respiratory illness, idiopathic pulmonary hemosiderosis, skin, G.I. tract, fatigue, neuropsychiatric symptoms, cancer, reproductive effects, and rheumatologic and other immune diseases.

Subsequent monographs of peer reviewed publications have been published (71, 72). These two monographs feature independent research on subject of indoor mold, water damage and human health. The various authors have clearly used scientifically and medically accepted methodologies to come their conclusions. The health problems attributed to verified exposure to these indoor environments include, but not limited to, multiple organ symptoms, asthma (adults and children),

pulmonary impairment, building related illnesses, neurocognitive deficits, central and peripheral neurological injury, autoantibodies (neural antigens and ANA), immune alterations (activation and suppression), changes in quantitative electroencephalogram, antibodies to molds and mycotoxins, and chronic fungal sinusitis. The statistical analyses presented in several of these papers demonstrated significant health problems with confidence intervals ranging from 95 % and greater (p values from 0.05 to 0.001). Finally, several other peer reviewed research papers published after the cutt off date of the IOM Committee confirm and extend the observations published in these two monographs (73, 74) as well the observations in research papers previously cited (5, 21, 26, 32-35, 39, 54).

Currently, technology does exist to determine total indoor particulate mycotoxins, mycotoxins in urine and body fluids as well as real time PCR DNA tests for fungi in human tissues (biopsy and pathology specimens) from exposed ill patients. For further information regarding these technologies contact the senior author of this publication.

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