Mycotoxins: Critical Information for Mold Remediation Contractors and Occupants

Abstract
The purpose of this paper is to share critical information with mold remediation contractors and occupants of structures with fungal contamination regarding the importance of addressing mycotoxins during evaluation and remediation. Mycotoxins, the chemical byproducts produced by mold, are linked with many health effects and need to be removed, as well as the visible colonies and microscopic spores.

The paper progresses logically from a review of the current approach to mold remediation procedures and is followed by more detailed discussion of mycotoxins and their health effects. The controversial elements of what role mycotoxins play in the symptoms of individuals in water-damaged buildings are buttressed with information related to mycotoxin exposure routes and some of the known differences between short-term and long-term exposures. A comparison of common testing procedures to assist contractors and occupants in evaluating the effectiveness of mold remediation projects is offered, which incorporates information on mycotoxin testing, as well as spore trap and polymerase chain reaction testing.

Incorporating mycotoxin testing more regularly into assessments of water-damaged facilities, particularly where occupants are complaining about health effects that could be related to the environmental conditions, has a variety of important benefits. One of the main advantages of mycotoxin testing as compared to other types of air or surface samples, is that such tests can more definitively link the poisons recovered from the environment to poisons recovered from medical samples from the occupants. Evaluating this potential connection helps to clarify whether the primary remediation effort needs to be directed toward cleaning up the environmental contamination, addressing residual contamination in the occupant's body, or both.

Mold Remediation Must Address Both the Visible and Invisible Contaminants
Many mold remediation contractors focus solely on the removal of visible contamination. While a visual cleaning standard is certainly of help in the initial phases of gross removal, it is often not a detailed enough "yardstick" to use to measure the effectiveness of a mold remediation project. This is especially true for the more complex or multifaceted projects; especially where sensitized individuals are occupants and the cleaning of microscopic contamination takes on greater importance.

Regardless of the sensitivities of the occupants, decades of experience in the mold remediation industry have indicated that a narrow focus on visual cleanliness can often lead to incomplete correction of the problems. This is one of the main reasons why the Institute of Inspection, Cleaning and Restoration Certification (IICRC) expanded the concept of what mold contamination was in their *Standard for Professional Mold Remediation* document. Rather than relying solely on visual evidence to determine mold contamination, the experts that developed the IICRC mold standard took the concept of a normal indoor environment not having visible mold, compared to a mold-contaminated environment where the colonies were visible, and addeda third option. Their definition of "Condition 2" environments notes that it is:

> *An indoor environment which is primarily contaminated with settled spores or fungal fragments that were dispersed directly or indirectly from a Condition 3 area and which may have traces of actual growth.*[1]

Even with this emphasis on understanding that a mold-contaminated environment can be more than the areas with visible mold, the S520 Standard drops back into the typical focus on visible mold in its discussion of contamination removal. The S520 notes that:

> *Remediated structures, systems, and contents can be considered clean when ……. surfaces are visibly free of dust.*[2]

Such a focus on visibly clean may suffice to alleviate some symptoms experienced by occupants which are related to water-damaged buildings, but sadly is often a disservice to the increasing number of clients that are mold-sensitized. Despite the obsession with only dealing with visible colonies of fungal growth, a thorough mold remediation effort should include the cleaning of areas and contents impacted by contamination through settled spores and fragments that are often unobservable without magnification or testing. By not addressing the full range of mold contamination, a contractor can leave themselves open to costly follow-up calls and the difficulties of dissatisfied customers who are still dealing with invisible, but real, contamination and health effects.

It is now clear that contamination from settled spores and fungal residues can cause serious lingering health effects to sensitized individuals. More importantly, exposure to fungal contaminants can lead to sensitization in individuals who were not previously experiencing ill health effects from mold. As noted by the Environmental Protection Agency:

> *Repeated or single exposure to mold, mold spores, or mold fragments may cause non-sensitive individuals to become sensitive to mold, and repeated exposure has the potential to increase sensitivity.*[3]

These microscopic contaminants can remain even if restoration efforts were otherwise determined to be effective based on a simple visual standard. If the “Condition 2” contaminants are not properly considered and addressed, the mold remediation project will not meet the current industry guidelines and pose additional risk for the occupants. Therefore, it is critical for contractors to understand the current science on microscopic fungal pollution and how it relates to proper mold remediation.

Mycotoxins as a Component of Microscopic Fungal Contamination

"Mycotoxin" is the term used for a variety of substances naturally produced by fungi. It is one class of chemical compounds that are formed as part of the growth process of fungal organisms, particularly mold. As the second part of the term mycotoxins suggests, these particular chemicals that come from mold are actually capable of causing disease and death in both humans and other animals. Other types of chemicals are also produced by fungi that are poisonous to plants (phytotoxins) and bacteria (antibiotics). Although these chemical molecules can play a role in protecting a suitable growth environment for the mold from other competing organisms, they may also serve other purposes, including aiding in the initial enzymatic break-down of surrounding food sources for easier consumption by the fungal colony.

Because of a wide range of factors (moisture level, pH, amount of light, temperature, nutrients available, growth phase of the fungal colony, the presence of competitive microflora nearby, etc.) appears to impact the production of the various fungal poisons, addressing mycotoxins in a contaminated environmental space can be even more vexing than the issue of dealing with microscopic spores. The growth of fungal colonies will eventually lead to the production of spores visible to the naked eye, but not necessarily to mycotoxin production. The EPA explains this variation in why mycotoxins are sometimes present and other times absent in their mold training course:

> *The amount and types of mycotoxins produced by a particular mold depends on many environmental and genetic factors. No one can tell whether a mold is producing mycotoxins just by looking at it.*[4]

Even when the focus of a proper remediation project is on both the visible and invisible contamination, there is a legitimate question of how mycotoxins fit into the picture. As noted previously, many industry guidance documents indicate that proper removal involves the elimination of visible growth, fungal spores, and fragments. Only recently (as more information becomes available regarding the potential adverse effects of residual mycotoxins in buildings impacted by mold), has the remediation industry taken a second look at what role mycotoxin cleaning should play in the remediation project.

Additional studies have found that of all the contributing environmental factors, mycotoxin production in buildings appears to be most closely related to the extent and frequency of water damage. A noted paper from the mycology group at the Technical University in Denmark documented research, which showed that mycotoxin production from mold colonies accelerated as surfaces got closer to the saturation point. They theorized that the additional moisture was necessary for the production of the mycotoxins as they are excreted in a liquid form. The group also showed that the mycotoxins can adhere to the fungal spores and other parts of the mold organism, even after the moisture source dissipates and the colonies dry down. These findings have very practical applications to occupants in water-damaged buildings and the remediation contractors working to address problems that impact both the structure and the occupants.

> *For this reason, the worst-case scenario for the development of an indoor mold problem involves a series of water intrusion events that allow large quantities of biomass and mycotoxins to form, then a period of drying that promotes the dispersion of spores and colony fragments, followed by their deposition throughout the building.*[5]

The Danish study, and others, correlate the expansion and contraction of the water supply with conflict between strains of fungus. This competition for resources results in an increase in the fungal defensive mechanism of mycotoxins. In addition, as water availability increases, fungal colonies grow. Any subsequent decreases in the amount of available water can spur the mold colony into releasing as many spores as possible as a survival technique; accelerating a process known as sporulation. This is the microbial equivalent of packing up the tent in search of greener pastures.

When this dispersion occurs, many of the mold colonies releasing high spore counts will also be liberating a large volume of fungal fragments. This fact has been documented by numerous studies with one publication showing that:

> *Aerosolized fungal particles in chamber studies have shown that fungal fragments are released at levels up to 514 times higher than spores.*[6]

The release of fungal fragments in conjunction with spores is important in the discussion of mycotoxins, as the excreted poisons do not stay exclusively on the surface but also surround all the different parts of the mold colony, including the hyphal fragments and spores. In situations where mycotoxins have been produced, this leads to the now distributed spores and particulates carrying a substantial mycotoxin load with them to their new landing place.

Mycotoxins and Health Effects

An understanding of the natural causes of mold growth and mycotoxin production is useful in demonstrating why the proper remediation of mold contamination from water-damaged buildings must move beyond the visual clearance criteria. Nevertheless, the more critical use of this information for the restoration contractor and the sensitized individual is in developing an appreciation for the health effects that these mold-related poisons can have on the human body.

Although a significant body of research related to mycotoxins and health effects has been developed over the last 20 years, there are still a lot of unknowns. Part of the difficulty in understanding all of the specific health effects from mycotoxin exposure, is the sheer number of poisonous compounds that have been identified related to fungal materials. The EPA notes that:

> *More than 200 mycotoxins from common molds have been identified, and many more remain to be identified.*[7]

In that same document, the EPA goes on to state that:

> *Some mycotoxins are known to affect people, but for many mycotoxins little health information is available. Research on mycotoxins is ongoing. Exposure to mycotoxins can occur from inhalation, ingestion and skin contact. It is prudent to avoid unnecessary inhalation exposure to mold.* [8]

The EPA's generally cautious approach to mycotoxins is buttressed by a number of other organizations and studies. The World Health Organization (WHO) notes:

> *The adverse health effects of mycotoxins range from acute poisoning to long-term effects such as immune deficiency and cancer.*[9]

One of the primary difficulties in pinning down the specific mycotoxin-related health effects of occupants is that the mycotoxins are not produced in isolation. A damp environment, particularly one related to a water-damaged building, proves to be a great breeding ground for a number of biological contaminants, not just mold. Bacteria, fungus, and even viruses can not only survive, but reproduce on damp or wet materials. Many of these organisms are beneficial to humans when they are part of natural processes to break down organic waste into recyclable constituent components. In contrast, being exposed to such organisms in the high concentrations and protracted durations created by the amount of time people generally spend indoors can contribute to greater incidences of negative health outcomes for the occupants. This reality of the harmful effects of living in water-damaged buildings is confirmed by numerous national and international agencies (including the National Institute for Occupational Safety and Health, the Centers for Disease Control and Prevention, the World Health Organization, Britain's National Health Service, and many others). All of them have put out information warning of the possible ill health effects from occupying damp and moldy environments.

Although the impact of these multiple organisms growing in the same place will contribute to the production of an increased level of mycotoxins from the fungal colonies, it will also result in numerous other materials and compounds being produced, which can also have a direct impact on the occupants' health. The World Health Organization in their 2009 advisory publication entitled WHO *Guidelines For Indoor Air Quality: Dampness And Mould* offered reasonable caution in their discussion of the sorts of indoor contaminants that can cause problems in water-damaged buildings.

> *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.*[10]

The Mycotoxin Controversy
Despite this consensus that water-damaged buildings can be harmful, there is not nearly as much agreement regarding the exact causes of the problems. While researchers know that microbial growth in a building can hurt people in a variety of ways, the exact process of how the illnesses occur still eludes scientists. It is well reported and accepted that the negative health impacts of mold exposure can develop from four different mechanisms: allergic reaction, invasive infections, respiratory irritation from the gases and chemicals released by growing colonies, and poisonous effects from the mycotoxins. The difficulty is in sorting out which forms of mold exposure, or combination of the four mechanisms, causes a specific symptom experienced by mold-exposed individuals. Answering this question is the subject of ongoing research and debate.

The discussion is further complicated by considerations regarding individuals that have underlying health issues (particularly immunocompromised individuals versus those with a normal immune system referred to in the medical literature as "immunocompetent"), and whether sensitization to mold or other substances has occurred. An additional factor to consider is the route of exposure of potential mold contaminants. Are individuals adversely impacted by mold contaminants and mycotoxins only when they ingest large quantities through contaminated foodstuffs, or is inhalation and skin contact with mycotoxins an issue as well?

The role of mycotoxins in the big picture of people suffering health effects from water-damaged buildings has been investigated for decades. As early as 1986, Harriet Burge, one of the first industry leaders in the modern understanding of fungal contamination, developed a risk assessment for reviewing indoor mold contamination situations. This risk assessment incorporated the known allergic and poisonous characteristics of mold from mycotoxins available at that time. Burge's model, primarily based on calculations of how much mycotoxins would be inhaled by an individual in a mold-contaminated structure, indicated that most indoor mold growth is not extensive enough to result in a level of mycotoxin inhalation sufficient to produce harmful health effects.

This assessment that inhalation of mycotoxins from mold-contaminated environments could not produce serious illness was challenged dramatically in the fall of 1994. Over just a few weeks, 10 infants from the eastern inner-city area of Cleveland arrived at one hospital with acute pulmonary hemorrhage (bleeding from the lungs). Dr. Dorr Dearborn, a pediatric pulmonologist, was the primary treating physician and worked with the Centers for Disease Control and Prevention to identify the cause of so many similar illnesses. The health specialists found that nearly all of the families lived in water-damaged homes that contained *Stachybotrys* mold.[11] The medical professionals determined that the infants had inhaled the toxic spores, which investigators associated with the acute pulmonary hemorrhaging. Many other anecdotal reports in the following decades supported the plausible connection between mold exposure and bleeding lungs.[12]

Mycotoxin Exposure Routes

In the years since Dr. Burges' 1986 model calculations indicated that inhalation of mycotoxins by individuals living in a mold-contaminated environment would not be enough to cause significant health effects, this assessment has been challenged on a number of fronts. Many of the peer-reviewed studies regarding potential mycotoxin exposure were reviewed as a basis for an article written for the layperson that was directed at the mold-sensitized individuals entitled: *The Mystery of Mycotoxins in Mold Contamination*.

In that article, the authors took a much broader approach in reviewing the existing information, something that is difficult to do in closely controlled medical studies where the researchers are trying to limit the number of variables that could impact the results. The blending of common sense and science led the authors to look at the evaluation of the dangers of other hazardous materials. This wider approach to the issue resulted in the consideration of mycotoxin exposure from multiple entry points into the body as summarized in the text:

> *As the information from calculated potential exposure amounts was studied more closely, concepts from allied fields were used as a better model. In particular, the concept of "total body burden" developed for lead dust exposure was seen as being applicable to mycotoxins, as well. Mold remediation professionals and ill occupants understood that living in a water-damaged structure resulted in numerous types of potential exposures—not only inhalation of airborne spores and mycotoxins. As the lead remediation professionals learned, small particle contamination in a house results in ingestion and possible skin absorption as well as inhalation. All three potential routes of entry must be evaluated in order to get a true picture of the potential risk.*[13]

Even the closely controlled study in Cleveland related to the infants with bleeding lungs did not resolve the controversy regarding mycotoxin exposures. Later information from the Center for Disease Control and Prevention tried to walk back the identified connection between the bleeding lungs and the presence of *Stachybotrys*. Since the primary mycotoxin produced by *Stachybotrys* is a compound known as trichothecenes, and those chemicals are proven to cause bleeding lungs when ingested on contaminated food, the determination by the government agency to cast some doubt on the findings appear to many individuals in the restoration industry to be politically motivated.

In contrast to the CDC's hesitancy to confirm a connection between *Stachybotrys* and significant health effects, multiple researchers continue to explore why such a dramatic health symptom would seem to be associated with a specific type of mold, rather than mycotoxin exposure in general. Such detailed studies resulted in multiple documents having conclusions similar to this:

> *Since the modes of activity of trichothecenes differ from aflatoxins and other mycotoxins, the combinations of mycotoxins could be additive or even synergistic, which could significantly reduce the amount of either mycotoxin required to induce an immunosuppressive or toxic effect. Immunosuppressive and combinational effects of multiple mycotoxins may constitute major components in the adverse health effects reported by many of the victims of mold exposure.*[14]

The mere presence of mycotoxin-laden spores, or the existence of the proper water damage conditions to accelerate their growth, does not mean that every occupant of the building will be exposed to dangerous chemicals from mold or suffer ill health effects. However, occupying water-damaged buildings has been definitively connected with adverse health effects, and the impact of mycotoxin exposure cannot be ignored.

While mycotoxins can be released as a vapor during the growth cycle of a fungal colony leading to direct inhalation, there is also substantial risk of inhaling large quantities of these hazardous compounds, which are affixed in the form of a viscous liquid to the spores that become airborne. Those same chemicals can be absorbed through the skin or ingested when they settle on food or drink.

The University of Connecticut Health Center has a special division that looks at indoor environmental connections to human health. In order to put together a guide to help physicians understand mold exposures and disease, the medical professionals conducted a meta-review of available medical literature. The result was a comprehensive publication entitled *Guidance for Clinicians on the Recognition and Management of Health Effects Related to Mold Exposure and Moisture Indoors*. In that document, the researching physicians looked at both theoretical and practical implications of treating patients with symptoms that may be related to exposure to water-damaged and mold-contaminated buildings. Part of the research evaluated the level of exposure to contaminants, such as mycotoxins, which would be enough to trigger symptoms. While recognizing the myriad complications in evaluating such situations, the University of Connecticut document noted that mycotoxin-related health effects from occupying a water-damaged building are certainly possible.

> *These, or even more complicated, considerations revolve around the situation that obtains during exposures to a "wet building" with chronic mold growth and low-level exposures to fungal allergens, volatile organic compounds, and mycotoxins, with resultant occupational diseases or residential "building-related disease." In these cases, the patient may suffer chronic exposures to mycotoxins, combined with other co-factors, one or more of which may be at dose levels at or fluctuating around the threshold for adverse effects.*[15]

A more definitive assessment of the potential for illness from mycotoxins was provided in a medical study entitled *Adverse Health Effects of Indoor Molds.* In that article, the authors laid out substantial information supporting the view that many doctors are not performing an adequate assessment of the potential environmental factors impacting their patients, particularly in regards to potential mold exposure. As such, they were blunt in their conclusion that mold-related illnesses are not only a danger for individuals with sensitivities over pre-existing conditions with compromised immune systems, but is a real risk for even "healthy" individuals.

> *Failure to perform the appropriate objective evaluations on patients may account for the commonly held belief that indoor mold exposure poses no significant health risks to immunocompetent humans. Conclusions: Exposure to high levels of indoor mold can cause injury to and dysfunction of multiple organs and systems, including respiratory, hematological, immunological, and neurological systems, in immunocompetent humans.*[16]

Short Term and Extended Exposures to Mycotoxins

As laboratory and medical studies have expanded over the past two decades since the issue of mycotoxin exposure first entered the public consciousness because of the ill babies in Cleveland, other details regarding mycotoxin exposure and disease have been clarified. One critical takeaway from this growing body of evidence is the cumulative nature of exposure to mycotoxins.

This is especially true for individuals who are sensitized to mold or have genetic conditions that do not allow them to naturally remove the mycotoxins from their system. In one specific study[17] the research team saw similar, if not nearly identical, results between two groups of exposure regardless of whether the mycotoxins happened through a single large dose or the same dose was spread across multiple days. This result reinforces what many veterans of the restoration and industrial health and safety industry have known for a long time. Namely, acute and long-term exposure can both lead to heightened sensitivity and poor health outcomes.

Specific Symptoms Related to Mycotoxin Exposure, Including Neurological Effects

The confirmed assertion that exposure to water-damaged buildings, and the mycotoxins in them, can lead to ill health effects begs the question; what might those effects be? Numerous publications provide a range of accepted symptoms related to exposure to mold in a water-damaged building. The EPA's mold training course, discussed previously, includes a summary of mold-related health effects:

- Headache
- Sneezing
- Runny nose
- Red eyes
- Skin rash (dermatitis)

- Increased asthma attacks
- Irritation of eyes, skin, nose, throat, and lungs
- Hypersensitivity pneumonitis
- Opportunistic infections

In a similar fashion, the guidance document on mold and mycotoxins for physicians published by the University of Connecticut Health Center lists these specific symptoms occurring in patients living or working in buildings with mold exposure:

- chronic respiratory complaints
- eye and skin irritation
- fatigue
- multisystem complaints (including inflammation of the upper and lower respiratory tract, skin, and mucous membranes, along with central nervous system symptoms such as headaches, nervousness, difficulty concentrating, dizziness, and excessive fatigue)

From just these two summaries, nearly everyone from the layperson on the street to the medical doctors prescribing treatments are aware that mold, along with the many other abnormal environmental aspects of a water-damaged building, can cause allergenic effects. Mostpeople will readily accept that mold exposure will lead to a stuffy nose, sneezing, and perhaps irritated eyes. What is much more difficult for most people to appreciate is the intensely debilitating symptoms suffered by a small minority of the population; particularly when the exposure is considered to be mild or other exposed individuals do not show the same magnitude of illness.

A common complaint from mold-sensitized individuals is that the general public does not appreciate the variety or intensity of symptoms that can afflict someone impacted by fungal contamination. This appears to be related, in part, to the fact that many of the cases of mold and mycotoxins contamination illness get diagnosed as something else. For example, the research team in a 2013 paper entitled *Detection of Mycotoxins in Patients with Chronic Fatigue Syndrome* (noted in the report as CFS) concluded that:

> *Mycotoxins can be detected in the urine in a very high percentage of patients with CFS. This is in contrast to a prior study of a healthy, non-WDB exposed control population in which no mycotoxins were found at the levels of detection. The majority of the CFS patients had prior exposure to WDB. Environmental testing in a subset of these patients confirmed mold and mycotoxin exposure. We present the hypothesis that mitochondrial dysfunction is a possible cause of the health problems of these patients. The mitochondrial dysfunction may be triggered and accentuated by exposure to mycotoxins.*[18]

The findings of this research may, with further confirmation, prove to be life-changing for many people who suffer from this debilitating lethargy. If mycotoxins, a controllable foreign substance, are responsible for the draining and destructive effects of chronic fatigue syndrome for even a portion of the individuals with such a diagnosis, then there is a proven action plan to help overcome the disease. This approach, similar to that utilized with other known environmental contaminants, integrates medical corrective actions with improvements in the environment to eliminate (or at least substantially reduce) continued exposure to the mold and mycotoxins.

Nor is the study that connected mycotoxin exposure with chronic fatigue syndrome the only such document. A 2018 meta-research effort evaluated over twenty other studies looking at fungal-related health effects. The study was a comprehensive effort to correlate patterns across multiple independent groups in the medical and environmental fields. The goal of the research was to better understand the health effects related to mold and mycotoxins. Although it did not have all the answers, the study should put to rest the idea that some individuals who suffer from a wide variety of mold-related illnesses are just "crazy" or suffering from a psychosomatic condition where they just think they are sick. The research conclusion should be shared widely in the restoration industry to assist mold remediation professionals in appreciating the serious health problems that some people suffer when exposed to mold and mycotoxins.

> *Exposure to mold and their mycotoxins continues to be a major health problem worldwide. Recent studies have greatly expanded our understanding of the systemic impact of mold toxicity on the human body, including the brain. Exposure to mycotoxins has demonstrated positive associations with asthma, wheezing, and bronchitis, as well as fatigue, musculoskeletal pain, headaches, anxiety, mood, cognitive impairments, and depression. A better understanding of the molecular pathways that underlie the link between mycotoxin exposure and cognitive impairment, as well as the impact of mold and mycotoxins on the immune and nervous systems, is urgently needed.*[19]

Although not doctors, thousands of professionals in the restoration industry that have dealt with mold remediation for over two decades have seen the actual impact of fungal-contaminated buildings on certain occupants. The individual and combined experience of these professionals kept pushing them back to the simple conclusion that exposure to mold-contaminated environments can produce a wide range of debilitating symptoms. With so many consistent factors lining up and pointing to the toxic nature of mold leading to severe health effects, the long-held suspicions of mold remediation industry contractors, consultants, and experts are steadily being vindicated by the medical community. Just as importantly, the same experience indicates that there are proven methods that can be used to make the environment better for such impacted individuals.

Basics of Mold Sampling
Traditional tape-lift and spore trap samples have been the ‘go-to’ testing method of the restoration industry for over two decades. These common sampling methods collect fungal spores out of the air or from surfaces on a sticky substance. The samples are then stained and looked at under a microscope to identify spores, pollen, and fungal fragments.

Up until the late 1990s, mold sampling was laborious and expensive. Culture plate sampling was the most reliable method. Specific sampling devices were utilized to draw suspected air contaminants onto specific types of nutrients in a Petri dish. These dishes had to be incubated so that mold spores embedded in the wet material could actually grow into fungal colonies. Then the colonies were visually examined and subjected to a number of chemical tests to determine the contaminant down to the species level. This methodology, which originally was developed to identify airborne bacteria, has three specific drawbacks when used for mold:

1. The amount of time that it takes to culture the sample (7+ days).
2. The number of Petri dishes of different nutrients that are necessary to get a full picture of the types of molds in the air.
3. The fact that only viable spores (those that are undamaged and able to grow) are represented in the results.

The move to spore trap sampling as compared to culturing the samples was based on the simplicity of the sampling and substantially faster analytical process. However, physical examination of spore types under a microscope are limited to identifying molds to the genus level. Additionally, when trying to connect the environmental factors of the building with the health situation of an occupant, spore trap sampling offers limited help in determining the toxicity of what is actually inhaled and ingested. Another drawback is that spore trap type sampling methods only offer a snapshot in time as compared to a longer view of the occupants’ exposure. These limitations mean that spore trap sampling is often of limited value in determining specifically what contaminants may be triggering the symptoms of a particular occupant.

These limitations in spore trap sampling by no means exclude these methods from ever being useful. Ask many sensitized individuals and they will be able to tell how their situation was alleviated or resolved with information gained by collecting just such samples. Still, for some occupants suffering from mold-related illnesses, successive rounds of spore trap samples can become an exercise in frustration, as even tremendously clean results on such samples leave them dramatically ill.

Even so, it is also important to point out that many individuals who suffer from mold-related illnesses do not understand that medical treatments to deal with the built-up toxins in their system must go hand-in-hand with the environmental cleanup if they are going to return to full health.

Mold Sampling to Assess Interior Conditions as They Impact Health

While cultured samples and spore trap samples still provide many benefits to the restoration industry and occupants that may be inhabiting water-damaged/mold-contaminated structures, additional efforts were made to address some of the weak points of those methodologies. In 2006, researchers from the Environmental Protection Agency (EPA) and the Department Of Housing And Urban Development (HUD) performed a comprehensive visual inspection and collected a variety of samples from 1,083 randomly selected houses across the United States. A major part of that effort was to identify the percentage of water-damaged homes, determine how water-damaged homes impacted asthma rates, and see if there was a simple test that could substitute for the extensive visual inspection and interviews in order to determine if the home had lingering effects of water damage.

As part of that process, the researchers used a specific vacuum sample technique for carpeted surfaces in the living room and main bedroom. The dust samples from those homes were analyzed using a DNA-based technology called mold specific quantitative polymerase chain reaction (PCR) (MSQPCR). That analysis was specific to 36 "indicator" mold species, which assisted the researchers in determining what houses were water-damaged. Because they compared 26 of the mold types that are typically found in such homes to 12 types of molds which typically enter a house through natural infiltration of outside air, this entire process was then dubbed the Environmental Relative Moldiness Index (ERMI). To interpret the results, the ERMI scale ranges from approximately −10 to 20 (low to high). The upper quartile (highest mold contamination quartile) starts at an ERMI value of approximately 5.

By 2013, the ERMI process of identifying water-damaged homes and subsequent asthma in children was well validated. One report published in the March 2013 Journal of Asthma included this conclusion:

> *High ERMI values were associated with homes of asthmatic children in three widely dispersed cities in the United States.*[20]

Shortly after the introduction of the ERMI sampling system, components of that were being used to evaluate the appropriateness of fungal remediation projects. Assessors and contractors started sending samples to labs to be analyzed using the PCR-MSQPCR technology. Samples that came back with low ERMI scores were judged to be completed properly. However, a major change had worked its way into the process. Instead of using a vacuum sampler to collect dust from two specific carpeted areas in the home, microfiber cloths in the form of Swiffer dust wipes were being used to wipe a large number of horizontal surfaces from multiple areas of the home.

Despite the lack of precision in this alternate sampling method, and without validation showing that the two sampling methods were equivalent, the sample results were being compared to the original ERMI scale. The discrepancies with ERMI samples being misused (particularly since the process was not only developed by the EPA, but also licensed to various laboratories) were significant enough that the EPA Office of Inspector General actually investigated the situation. One of their main conclusions was blunt in its common sense understanding of the situation:

> *If mold samples are not collected in accordance with the sampling procedures used to develop the ERMI, the results would be of questionable value.*[21]

The difficulty of using ERMI scores to determine the successfulness of mold remediation projects, especially for people sensitized to mold, did not stop others from trying to harness the power of the new analytical technique. One of the most well-known proponents of utilizing the ERMI sampling technique to assist patients in identifying whether their residences were safe for occupancy is Dr. Ritchie Shoemaker.

However, rather than trying to force the ERMI scoring system that was designed to identify water-damaged buildings, Dr. Shoemaker conducted several large studies with patients and identified five specific mold types identified in ERMI samples, which seem to have an outsized impact on his clients. Dr. Shoemaker used this information to develop his HERTSMI-2 rating system (Health Effects Roster of Type-Specific Formers of Mycotoxins and Inflammagens – 2nd Version, cleverly named to sound like "hurts me" when spoken aloud). This completely separate scoring system gives a range of point values to five specific mold types that are thought to cause the greatest problems for sensitized individuals. On his website, Dr. Shoemaker explains the rationale for choosing the five mold types.

> *We selected five species of fungi from Group I of ERMI based on two criteria.*
> *1. Representative of varied water saturations (60-80%; 80-90%; 90-100%).*
> *a. Relative risk for enrichment is WDB compared to non-WDB is at least 10.*[22]

Mycotoxin Sampling as the Next Major Advancement in Mold Investigations and Remediation

Given the history and limitations of cultured and spore trap samples for mold, there was a clear need for more sensitive testing compared to what spore trap analysis can provide. The big step of ERMI style samples moved environmental sampling to a closer connection to occupant health effects. Still, eight years later, the Swiffer cloth-style sample collection has never been rigorously validated. This may explain some of the difficulty in tying occupant symptoms to specific environmental conditions, particularly for projects where the occupants self-identify as sensitized individuals, or a significant range/severity of symptoms is reported.

As the understanding of the impact of fungal contamination reached further into both the medical community and restoration industry, there was a growing need for testing options to determine the exposure of building occupants to mycotoxins. This gap was bridged with the introduction of the Environmental Mold and Mycotoxins Assessment (EMMA) sampling system. The EMMA test uses the newer analytical techniques to identify and quantify 12 mold species. The 12 mold types are known to produce the mycotoxins confirmed by various scientific studies to be some of the most dangerous to human health. To complement that analysis of mold spores and fragments, the same EMMA sample is used to identify 16 of the most poisonous mycotoxins.

The selection of the mycotoxin panel included in the EMMA samples is a result of research, which identified the types of mold-created poisons that most commonly create symptoms for individuals occupying a water-damaged building. This mycotoxin analysis allows the results from the EMMA samples to be correlated directly to biological samples to determine if the poisons in a person's system are likely to be coming from their place of occupation. A simple urine test provides the mycotoxin information to match with the EMMA results. Because specific mycotoxins recovered from the built environment can be compared to the types of fungal poisons obtained from biological samples collected directly from people, both building remediation efforts and individual medical treatments to be targeted much more precisely.

The ability to sample for mycotoxins from a particular structure and then compare with mycotoxin levels in the body of the ill individual brings clarity to both ends of the process that is needed to help sensitized individuals recover in a meaningful way from serious mold exposures. In a paper published in 2016, mycotoxin researchers provided clear evidence that:

> *Mycotoxins, specifically trichothecenes, aflatoxins, and ochratoxins, can be detected in human tissue and body fluids in patients who have been exposed to toxin producing molds in their environment. The toxins can be best determined in urine as a screening qualitative test which can assist the physician to determine what the best mode of therapy would be.*[23]

ATTACHMENT 1 summarizes a number of important details, which helps to explain the differences between ERMI, HERTSMI-2 and EMMA samples.

Practical Applications of Mycotoxin Testing

Unlike traditional spore trap sampling, mycotoxin sampling methods represent the long-term fungal load rather than a short window of time reflected in the standard ten-minute spore trap air sample. While it is true that a healthy body will process out some of the fungal toxins and other contaminants over time, the lag between exposure and complete contaminant removal in even the fittest of individuals still allows valid comparisons between people and structures.

This measurement of a long-term impact is a powerful tool in understanding the situation of a sensitized individual, or even just the occupants of a “normal” water-damaged building. By specifically measuring the total body burden and comparing those internal poisons to the environmental mycotoxin levels, both the health professional and the mold remediation contractor can move to directly address the specific issues of the structure.

For example, numerous sensitized individuals have seen success in regards to their remediation efforts only after they started completing their post-remediation verification testing with more sensitive DNA-based mycotoxin testing in conjunction with spore trap sampling. These tests can detect if the cleaning was completed successfully down to a level of precision that is difficult to achieve with microscopic analysis. The mycotoxin testing also avoids the logarithmic oddities in interpreting the data that can create false results that have been documented when using DNA-type mold tests like the Environmental Relative Moldiness Index (ERMI) samples. In fact, as a study conducted by the Texas Technical University pointed out:

> *...spore counts do not adequately represent the amounts of fungal fragments that are present in the air at any given time. In fact, fragments and particles that are the same size greatly outnumber intact fungal spores.*[24]

As with any technology, mycotoxin testing should be considered a tool, not a magic bullet. Incorporating mycotoxin testing will enhance existing sampling methodologies, not necessarily supplant them. For example, mycotoxin samples may not provide the same level of specificity as to the location within a property of problem fungal growth areas that spore trap sampling can achieve. As such, using mycotoxin sampling in conjunction with spore trap samples and a thorough visual inspection will often be necessary.

Still, mycotoxin testing of the occupants provides the highest level of specificity regarding what is actually impacting the people occupying a home or facility. Understanding this exposure can greatly assist homeowners, facility managers, and restoration contractors in making further testing and repair decisions. In addition, adding mycotoxin testing to the battery of available options open to assist the recovery of sensitized individuals can help the physician and patient connect the dots of all the myriad factors impacting the patient's health to find the proper medical path forward.

Conclusion

Understanding the value of mycotoxin testing is a powerful tool for the mold inspector and remediator to gain a greater understanding of the specific situation of a building or lifestyle. Of particular value is the ability for mycotoxin testing to be revolutionary when it comes to identifying the particular building where an ill occupant is being exposed (*i.e.,* home, office, main residence, vacation home, etc.).

Another critical use for the mycotoxin testing technology is for contractors and patients to use a series of samples to confirm that the exposure to harmful poisons in a property following mold remediation has been eliminated. Mycotoxin sampling can assure that the work was completed with a level of thoroughness that is not possible with a visual inspection or spore trap samples alone. Such clarity of data protects the contractor from lengthy call-backs and provides confidence that the work has created an environment that is appropriate for the client to live and heal in.

In addition to the inherent confidence-building nature of using multiple tests to provide mutually supportive conclusions, there is value in using a testing method that provides the specificity of a medically significant and trackable body burden. As contractors and physicians gain experience with the use of these sensitive tests, they are certain to become a critical tool in the complex process of creating the truly clean environments required to address the growing number of mold-sensitized individuals.

Endnotes

1. ANSI/IICRC S520-2015, *Standard for Professional Mold Remediation*, Third Edition, Section 3, Definitions, page 15

2. ANSI/IICRC S520-2015, *Standard for Professional Mold Remediation*, Third Edition, Section 4.4, Contamination Removal, page 18

3. Environmental Protection Agency (EPA), *Mold Course Chapter 1: Introduction to Molds*, Lesson 3 - Health Effects That May Be Caused by Inhaling Mold or Mold Spores https://www.epa.gov/mold/mold-course-chapter-1

4. Environmental Protection Agency (EPA), *Mold Course Chapter 1: Introduction to Molds*, Lesson 4 - Mycotoxins and Health Effects https://www.epa.gov/mold/mold-course-chapter-1

5. Technical University of Denmark; *Mycotoxin Production by Indoor Molds*; Kristian Fog Nielsenet al; The Mycology Group, BioCentrum; March 6, 2003 https://realtimelab.com/wp-content/uploads/2016/03/11.-Mycotoxin-production-by-indoor-molds.pdf

6. IntechOpen; *Fungal Growth and Aerosolization from Various Conditions and Materials*; Jacob Mensah-Attipoe and Oluyemi Toyinbo; April 30, 2019; https://www.intechopen.com/books/fungal-infection/fungal-growth-and-aerosolization-from-various-conditions-and-materials

7. Environmental Protection Agency (EPA), *Mold Course Chapter 1: Introduction to Molds*, Lesson 4 - Mycotoxins and Health Effects https://www.epa.gov/mold/mold-course-chapter-1

8. Environmental Protection Agency (EPA), *Mold Course Chapter 1: Introduction to Molds*, Lesson 4 - Mycotoxins and Health Effects https://www.epa.gov/mold/mold-course-chapter-1

9. World Health Organization, *Fact Sheet, Mycotoxins*; 9 May 2018 https://www.who.int/news-room/fact-sheets/detail/mycotoxins

10. World Health Organization; *WHO Guidelines For Indoor Air Quality: Dampness And Mould*; Page XIV https://www.euro.who.int/__data/assets/pdf_file/0017/43325/E92645.pdf

11. Archives Of Adolescent Pediatric Medicine; *Acute Pulmonary Hemorrhage in Infants Associated With Exposure to Stachybotrys atra and Other Fungi;* Ruth A. Etzel, MD, PhD, et al; August 1998; https://jamanetwork.com/journals/jamapediatrics/fullarticle/189798

12. EHS Today; *Mold and Bleeding Lungs: Another Connection? A Medical Doctor's Ordeal Leads Investigators On A Hunt For Mold Contamination*. Michael Pinto, Cherie Fennema; May 19, 2003 https://www.ehstoday.com/standards/osha/article/21909926/mold-and-bleeding-lungs-another-connection

13. Moldsensitized.com; *The Mystery of Mycotoxins in Mold Contamination*, Michael A. Pinto, CSP, CMP and Steve Levy, CIEC, CMC; July 16, 2015; https://www.moldsensitized.com/mycotoxins-in-mold-contamination/

14. Aerotech Monitor. 2000; *A Risk Assessment Model for Mycotoxin Producing Molds on Human Health in Indoor Environments*; Miller RV, Martinez-Miller C, Bolin V.; 3:4-5; page 4

15. University of Connecticut Health Center Division of Occupational and Environmental Medicine Center for Indoor Environments and Health; *Guidance for Clinicians on the Recognition and Management of Health Effects Related to Mold Exposure and Moisture Indoors*; Eileen Storey MD MPH, et al; September 30, 2004; page B-11 https://health.uconn.edu/occupational-environmental/wp-content/uploads/sites/25/2015/12/mold_guide.pdf

16. Journal of Nutritional & Environmental Medicine; *Adverse Health Effects of Indoor Molds*; September 2004;; Luke Curtis et al; https://www.researchgate.net/publication/253011596_Adverse_Health_Effects_of_Indoor_Molds

17. Toxicological Sciences; *Stachybotrys chartarum, Trichothecene Mycotoxins, and Damp Building–Related Illness: New Insights into a Public Health Enigma;* James J. Pestka, et al; April 2008; https://realtimelab.com/wp-content/uploads/2016/03/10.-Stachybotrys-chartarum-Trichothecene-Mycotoxins-and-Damp-Building-Related-Illnesses..pdf

18. MDPI Open Journal Toxins; *Detection of Mycotoxins in Patients with Chronic Fatigue Syndrome*; Joseph H. Brewer, et al; 2013, https://realtimelab.com/wp-content/uploads/2016/03/2.-Detection-of-Mycotoxins-in-Patients-with-Chronic-Fatigue-Syndrome.pdf

19. Clinical Therapeutics; *Effects of Mycotoxins on Neuropsychiatric Symptoms and Immune Processes;* Ratnaseelan, Aarane M. et al.; June 4, 2018 Volume 40, Issue 6, 903 – 917 https://www.clinicaltherapeutics.com/article/S0149-2918(18)30229-7/fulltext

20. Journal of Asthma; *Higher Environmental Relative Moldiness Index (ERMI) Values Measured in Homes of Asthmatic Children in Boston, Kansas City, and San Diego*; Vesper, Stephen et al; March 2013 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3874819/

21. US Environmental Protection Agency (EPA) Office of Inspector General; *Public May Be Making Indoor Mold Cleanup Decisions Based on EPA Tool Developed Only for Research Applications*.; Report No. 13-P-0356 August 22, 2013; https://www.epa.gov/sites/production/files/2015-09/documents/20130822-13-p-0356.pdf

22. SurvivingMold; *HERTSMI-2 Scoring System*; https://www.survivingmold.com/diagnosis/hertsmi-2

23. International Journal of Molecular Sciences; *Mycotoxin Detection in Human Samples from Patients Exposed to Environmental Molds*; Dennis G. Hooper, et al.; April 1, 2009; https://realtimelab.com/wp-content/uploads/2016/03/1.-Mycotoxin-Detection-in-Human-Samples-from-Patients-Exposed-to-Environemental-Molds.pdf

24. Department of Microbiology and Immunology, Texas Tech University Health Sciences Center; *Detection of Airborne Stachybotrys chartarum Macrocyclic Trichothecene Mycotoxins on Particulates Smaller than Conidia;* T. L. Brasel, et al.; August 16, 2004 https://realtimelab.com/wp-content/uploads/2016/03/8.-Detection-of-Airborne-Stachybotrys-chartarum-Macrocyclic-Trichothecene-Mycotoxins.pdf

ATTACHMENT 1

TEST TYPE / FEATURES	**ERMI** (Environmental Relative Moldiness Index)	**HERTSMI-2** (Health Effects Roster of Type-Specific Formers of Mycotoxins and Inflammagens – 2nd Version)	**EMMA** (Environmental Mold and Mycotoxins Assessment)
Who developed the test criteria and why?	**EPA -** to assist with identifying water damaged buildings.	**Dr. Shoemaker -** as an alternative interpretation of the ERMI results to assist sensitized individuals.	**RealTime Laboratories** -to identify the source of mycotoxins in buildings and correlate the data to occupant health/medical progress.
How many mold types does the test identify?	**(26)** molds that are common to water damage throughout the United States are compared to 12 types that typically enter from out-of-doors.	**(5)** leading targets that have demonstrated adverse health effects for sensitized individuals.	Identifies **(12)** selected mold types shown to create the highest levels of mycotoxins.
What is the purpose of the test?	To identify if a building has a history of water damage.	To determine if an environment is safe for a sensitized individual to enter.	To identify if mold or mycotoxins are present in the environment and/or the occupant.
How is the sample collected?	A sampling cassette is attached to a vacuum hose and passed repeatedly over a marked area of carpet for six minutes.	Various labs will analyze either vacuum samples like the ERMI or unscented Swiffer samples wiped over a large number of interior surfaces.	The environmental samples utilize a swab or gauze pad that is used to wipe a HVAC filter.
Does this test identify mycotoxins?	Not directly. The ERMI only demonstrates if a building is statistically likely to have a history of water damage.	Not directly. The HETSMI-2 test only looks for molds know to have health impacts.	Yes. In addition to identifying fungal types, the EMMA tests specifically identify 16 types of mycotoxins.
Does this test have a corollary medical test?	Not directly. The ERMI was developed to help identify if the environment might be contributing to asthma, but not to test for asthma.	Not directly. The HERTSMI-2 test is targeted to the presence or absence of a safe level of specific fungal types for sensitized individuals.	Yes. The mycotoxin types tested for in the EMMA test are directly related to the biological mycotoxins, which can be extracted from a urine sample.