



W O N D E R M A K E R S

E N V I R O N M E N T A L

Understanding And Dealing With Mycotoxins

Abstract

Mycotoxins are the name given to a large number of chemicals produced by mold as a means of protecting the colony from competing organisms. This paper describes how one chemical technology company became interested in mycotoxin research and solutions due to their wide-ranging impacts on human health. It goes on to describe the chemistry of mycotoxins that make them so difficult to remove with standard mold remediation techniques, a summary of some of the most significant health impacts of mycotoxins, and advances in testing techniques to identify the hazardous chemicals. The paper then offers test results displaying the efficacy of Superstratum Hypochlorous Acid Pro Cleaner against mycotoxins, followed by a recommendation for a revised mold remediation protocol that addresses mycotoxin contamination.

Introduction

The interest in mold and mycotoxins is growing rapidly, especially since mycotoxin contamination has been identified in a large percentage of water damaged and mold impacted buildings.^{1,2} As a producer of a number of chemical products for the mold remediation industry, the interest of the Superstratum team in understanding the role that mycotoxins play in occupant health was based on both commercial and personal motivations. The commercial aspect stemmed from the obvious desire to ensure that their remediation products and procedures were effectively addressing mycotoxin contamination as well as the mold itself.

Like many readers, each of the Superstratum team members have deeply personal experiences with mycotoxins, including family histories with chronic illnesses related to mycotoxin exposure. As the team delved deep into research of the health impacts of mold, the pattern became increasingly clear - individuals with a history of compounding chronic illnesses most all had one thing in common, a history of exposure to environments with water damage and mold. Unfortunately, the majority of their experience with doctors within centralized medicine failed to diagnose this as an underlying cause. The patients themselves often came to these conclusions from their own research before being accurately diagnosed and could begin the healing process.³ For instance, a significant amount of research around cystic fibrosis, a disease that impacted one of the team member's 3 siblings, indicated that fungal infections of the lungs were a major contributing factor.⁴ These life experiences keyed additional interest in sick building solutions.

The company was launched upon the discovery of the technology that would become the Superstratum Smart Polymer Coating (SPC). Given the company's background, the Superstratum team was looking to solve mold issues from a perspective that is focused on health and chemistry. Their efforts to find products that could potentially improve health outcomes by

resolving mold problems led them to a surprising revelation; mold was not the primary trigger for the compounding health issues, it was the mycotoxins associated with mold.

Mycotoxin danger is widely known in the food industry.⁵ The scientific literature showed that these toxins can enter the body through ingestion and skin absorption,⁶ but little research looked at the inhalation of mycotoxins as a possible route of hazardous exposure.

As the technology related to the sampling of mycotoxins in the environment improved, the recognition of their connection to ill health has increased as well.⁷ Several commercial laboratories⁸ now offer mycotoxin testing for environmental surface samples; and even air samples. Such samples support the biological tests for mycotoxins within the body through urine or blood tests which have been available for over a decade.⁹

Health Effects Associated With Mycotoxins

As noted in the previous section, health effects associated with mycotoxin exposure have been studied for over 100 years. The majority of this effort was focused on mycotoxins that were ingested as a result of eating mold contaminated food. In addition to the overall poisonous effects of the mycotoxins, organizations as diverse as the United States Department of Agriculture (USDA) and the World Health Organization (WHO) have recognized that exposure to these mold byproducts can cause a wider range of short-term and long-term effects. For example, the mycotoxins fact sheet provided by the WHO states¹⁰:

Mycotoxins can cause a variety of adverse health effects and pose a serious health threat to both humans and livestock. The adverse health effects of mycotoxins range from acute poisoning to long-term effects such as immune deficiency and cancer.

The number of possible health effects on people who are exposed to mycotoxins is substantial. In 2019, an article in the journal *Toxicological Research* provided a table with specific types of mycotoxins and the health effects that have been attributed to each particular substance through peer-reviewed evaluations.¹¹

Mycotoxin	Health effect
Aflatoxins	Hepatotoxic and immunosuppressive
Ochratoxin A	Carcinogenic, genotoxic, immunosuppressive, nephrotoxic and induction of upper urinary tract disease
Fumonisin	Carcinogenic, hepatotoxic, nephrotoxic, immunosuppressive
Deoxynivalenol	Nausea, vomiting, diarrhea, reproductive effects and toxicosis
Trichothecenes	Hepatotoxic, genotoxic, and immunosuppressive
Zearalenone	Carcinogenic, hormonal imbalance, and reproductive effects
Patulin	Neurologic and gastrointestinal

In addition to the liver, kidney, bladder, and gut issues pointed out in the above table, the fact that mycotoxins can lead to cancer and a weakening of the overall immune system means that mycotoxin exposure can create a base toxic overload which can make other health problems

more serious. Indeed, the number of specific neurological issues connected with mycotoxin exposure include severe headaches, eye pain, vertigo, tremors, difficulties with memory, cognitive issues, depression, anxiety, and obsessive-compulsive disorders.

Further research has connected mycotoxin exposure with a wide range of conditions including chronic fatigue syndrome¹², fibromyalgia, Alpha-gal Syndrome (a condition where individuals become allergic to consuming red meat after a tick bite or other toxic exposures), Postural Orthostatic Tachycardia Syndrome (POTS - a condition where too little blood moves back to heart when a person moves from a prone to a standing position)¹³, and multiple sclerosis¹⁴.

Many of the more severe health problems associated with mycotoxins may only be experienced by a portion of the population. This variation was as frustrating as it was puzzling until advances in understanding the genetic make-up of people provided a better explanation of the overall process of ill health that led to a wide variety of symptoms. As the medical understanding advanced, the primary points were summarized into layperson language and even shared in restoration industry trade journals¹⁵:

As the understanding of the medical aspects of mold sensitized individuals grows, it is becoming clear that a small, but substantial, percentage of the population have a genetic structure that does not allow them to break down mycotoxins in their system as efficiently as most people. In some of the more severe cases, hardly any of the mycotoxins are broken down by the person's immune system. This leads to a buildup of these poisonous chemicals in their blood and body systems. The poisons themselves, and their body's ineffective attempts to remove them, results in a number of illnesses.

The Fungal Connection: Mycotoxins As A Component Of Mold

As mold grows, the organism completes a number of chemical reactions to support life. This chemical process, at the cellular level, is what is called the metabolism of the mold. The primary purpose of mold metabolism is to convert food into energy and the building blocks for physical expansion of the colony. Anything created by the mold's chemical process that is not energy or a part of a growth structure is known as a secondary metabolite. Since mycotoxins are chemicals produced by many types of mold as a defense mechanism to protect the colony, they fit the classification as a secondary part of the metabolic process.

Currently, over 500 different types of mycotoxins have been identified.¹⁶ There are many factors that influence the production of mycotoxins. The most important consideration is the type of mold that is growing. It has also been shown that the same mold species can produce different mycotoxins depending on the specific circumstances surrounding the growth of the mold colony. Some critical environmental conditions that impact mycotoxin production include temperature, surface moisture, relative humidity, and availability of nutrients. In general, mycotoxins are produced by molds growing on wet surfaces with elevated temperatures and high humidity.

Since mycotoxin production is an essential defense mechanism for mold, the poisons may not be produced if there is no bacterial or fungal competitor for the nutrients where the mold is growing. One group of researchers were quite definitive when they stated: *Bacterial-fungal interactions influence mycotoxin production in addition to environmental factors*.¹⁷ In essence, the greater the threat to the mold colony from competing organisms, the more likely that those molds that have the ability to chemically create poisons will do so.

Mycotoxins Are Interesting Chemicals

The biological process of mold growth results in the production of a number of different types of chemicals from the organism. The secondary metabolites, which are non-volatile (*i.e.*, do not easily evaporate at room temperature), have a low molecular weight, and cause harm to people or animals are classified as mycotoxins.¹⁸ Though the mycotoxins are recognized as non-volatile, many are easily aerosolized when moving air passes over a fungal surface and pick up small particles with attached mycotoxins. In addition to protecting the mold colony from competing microorganisms, mycotoxins are thought to assist some parasitic molds in invading the tissue of a host.¹⁹

Although the chemical reactions happen inside the mold, the mycotoxins are exuded from the spores, hyphae, and mycelium. In other words, the poisons are emitted from many areas of the mold. The released mycotoxins are not easily destroyed, are not broken down by water, and do not absorb water.²⁰ Many of them have a sticky nature²¹ which allows the mycotoxins to cling to surfaces and airborne particles like dust and spore fragments. This means that they can be left behind even if the mold spores and fragments are removed during a cleaning process. Thus, mycotoxins are able to travel throughout the home in an aerosolized form, often infiltrating and being circulated by HVAC systems.

All of the critical properties of mycotoxins make them difficult to remove. The small size of the chemical droplets means that they are difficult to capture without dense filtration. The sticky nature of many of the mycotoxins along with their low molecular weight means that there is not enough mass for them to be separated from a surface by vacuum pressure. Their inability to be dissolved by water limits the effectiveness of many standard cleaning and antimicrobial chemicals. The fact that they do not evaporate in environments with temperatures in the normal living range results in their potential accumulation over time. If mycotoxins do get disturbed by the remediation or cleaning process, they may become aerosolized and migrate long distances before settling and sticking to other surfaces.

Identifying How Mycotoxins Migrate From Mold Sources And Impact Health

In both the health arena and the mold remediation industry it was suspected for decades that mycotoxins played a significant role in mold related illnesses of occupants in water damaged buildings. The theory was that the inhalation of the mycotoxins was providing a direct pathway into the body systems. It was further speculated that small amounts of mycotoxins could migrate directly from the nasal passage into the brain exacerbating the neurological problems experienced by many occupants in mold contaminated structures. (This direct assault on the brain through mycotoxins migrating through the nose was actually confirmed in animal studies.²¹)

While these speculations made logical sense, they were difficult to prove. As one study²² summarized it:

Although a large amount of attention has been placed on the presence of Stachybotrys mycotoxins in the indoor environment, attempts to identify these toxins in the dust usually fail, as do attempts to correlate amounts found on contaminated surfaces with amounts found in dust.

Nevertheless, the same research that clarified the difficulties in following the movement of mycotoxins in the environment showed that the mycotoxins migrated as independent small particles or as an attachment to other microscopic materials.²³

It was only in the last few years that an emphasis was placed on researching how mycotoxins could migrate, buildup, and then be spread in the environment. This newer research verified some of the earlier speculation about mycotoxins. A fascinating study proved that mycotoxins could be aerosolized from various substrates. Such research even went on to show that the aerosolization rates differed by fungal species. The research proved that it was possible that air speeds typically present in occupied buildings from the HVAC systems, and occupant movement, could aerosolize mycotoxins. It further showed a link between mycotoxin concentrations found on contaminated surfaces and in settled dust.²³ Specifically, the study emphasized:

Quantification of the toxic content revealed that the toxic load was mostly associated with particles of size $\geq 3 \mu\text{m}$. However, some ... can also be found on smaller particles that can deeply penetrate the respiratory tract upon inhalation.

When studies showed mycotoxins do move on particles smaller than 3 microns, the reality of deep penetration of the chemicals into the respiratory system,²⁴ as well as impact on the nasal system, was verified. The seriousness of negative impacts on brain health became more obvious as the recognition that mycotoxins penetrate through tissue^{19, 21} was added to the other findings.

Mycotoxin Sampling Procedures

Since mycotoxin testing for agriculture and food products has been a priority for decades, a number of different analytical techniques are available to identify, and even quantify, types of mold poisons. Livestock feed processors have had samples tested successfully for mycotoxins using monoclonal antibody isolation chemistry. In that industry, the less-expensive antibody testing has generally supplanted the more sophisticated, but significantly more expensive, techniques that use liquid chromatography with mass spectrometry.

For the environmental industry, enzyme-linked immunosorbent assay (ELISA) techniques have been developed for the identification of certain mycotoxins. While this technique is quicker and cheaper than many others, it must be set up to look for specific types of mycotoxins.²⁵ That is why many laboratories serving the mold remediation industry have a specific list of mycotoxins that they can identify. Currently, there is no industry recognized panel of mycotoxins that every lab should be identifying as possible threats to occupant health. This is a significant detriment to gaining a better understanding of the extent of mycotoxin contamination as a component of interior mold growth situations.

This sampling confusion is further magnified by the efforts to relate biological samples of mycotoxins utilized for diagnosis of health problems with the surface and air samples collected in a person's environment. Although the environmental sampling may have problems with which mycotoxins to evaluate and how to interpret the data, some of the biological samples are not even approved for doctors to use in the clinical evaluation of people who may have mycotoxin poisoning. In cases of suspect mycotoxin poisoning, blood samples are approved by the Food and Drug Administration for the purpose of clinical diagnosis.

However, over the past decade, a number of laboratories have begun offering services to identify mycotoxins in urine samples. While this avoids the difficulties and pain of a blood draw in the laboratory or hospital setting; according to the Center for Disease Control:²⁶

There is no FDA-approved test for mycotoxins in human urine.

Despite these challenges, there is some common-sense utilization that can come from collecting environmental and medical mycotoxin samples. For individuals who have symptoms that are consistent with mycotoxin exposure, a blood or urine test may help pinpoint which molds appear to be the problem. Follow-on air or surface samples looking for those specific mycotoxins can help to rule in, or rule out, whether mycotoxin residue could be contributing to the health issues. As is recognized with lead poisoning²⁷, in the cases where mycotoxins are found inside buildings, the effectiveness of medical interventions to detoxify the impacted individuals can be compromised if the source of the poison has not been removed.

It is also critical to understand that testing, by itself, is never the total answer. Even so, utilizing surface tests to determine whether chemicals used in the mold remediation field have a positive impact on mycotoxin removal will be a benefit to the cleaning and restoration industry. Such information would allow contractors and do-it-yourself practitioners to select the appropriate chemistry for the wet cleaning segment of a standard HEPA sandwich cleaning process.²⁸

A Process For Testing Chemicals For Effectiveness In Mycotoxin Removal

The Superstratum team had an existing chemical that they believed could be effective in removing mycotoxins from surfaces. The Superstratum Hypochlorous Acid Pro Cleaner was described and marketed as a cleaner rather than a disinfectant, so no EPA registration was required by the manufacturer or special restrictions or training required by the users.

The team engaged Wonder Makers Environmental to investigate the possibility of testing the effectiveness of the cleaner in removing mycotoxins from surfaces. The purpose of this testing was not to generate data for any sort of EPA certifications, but to answer a simple question frequently asked in the mold remediation industry: *"is there a cleaning product that removes mycotoxins from surfaces?"*

In response to the initial discussions, Wonder Makers proposed a "practical science" test designed to determine the efficacy of removing mycotoxins from surfaces. The test protocol was designed to evaluate the effects of two different aspects of the typical mold cleaning process. Of central importance is whether a chemical destruction of the mycotoxins occurs. Another theory that needed to be evaluated was whether the Superstratum product had an ability to separate the mycotoxins from surfaces so that they can be removed by wiping; which is a common cleaning process used as part of mold remediation procedures.

Overview of the Test Process

The test involved *Superstratum Hypochlorous Acid Pro Cleaner* in conformance with the manufacturer's instructions for restoration efforts. In section V of the Superstratum Pro System Manual the directions for using the *Hypochlorous Acid Pro Cleaner* are spelled out as:

- e. Suppress dust particles and possible spores by misting the air or fogging around the area with **Superstratum Hypochlorous Acid Pro**.*
- f. Start to remove damaged articles. Make sure to use a properly fitted P100 mask, protective suit, and protective gloves.*
- g. Clean away the mold by spraying with **Superstratum Hypochlorous Acid Pro**. Let sit for 10 minutes and wipe away or use a HEPA vacuum.*

With that background information as a basis for the experiment, the actual testing procedure evaluated the general efficacy of the Superstratum product in removing mycotoxins. The straightforward process involved testing the cleaner on an intentionally contaminated piece of

oriented strand board (OSB). OSB was chosen as the surface for the testing because it is a common building material.

Another important reason for using OSB for the initial test is that it has an irregular surface because of the various pieces or flakes of wood that are compressed to manufacture the sheathing. This uneven surface profile allows mold and mycotoxins to be partially embedded in the surface and makes the removal of contaminants more difficult. As such, if the Superstratum cleaner works to remove mycotoxins from OSB, its effectiveness can be expected to be even better on smoother surfaces.

Since the testing involved the application and disturbance of potentially dangerous mycotoxins, the process had to be completed within the confines of a biosafety level 1 laboratory. Therefore, Wonder Makers contracted with Medical Services Consultation P.A. (MSCPA); working with Real-Time Laboratories as a subcontractor.

Following the Wonder Makers' protocol, the actual testing involved several steps. Wonder Makers prepared three pieces of OSB so they were ready for contamination with mycotoxins and shipped them to MSCPA for preparation and sampling in their level 1 biosafety laboratory.

The *Superstratum Hypochlorous Acid Pro Cleaner* product was also provided to MSCPA by Wonder Makers to ensure that the manufacturer was not providing any material that was specially formulated for the test process rather than available for general sale. In addition, Wonder Makers also supplied the hand sprayers and rags to be used during the testing process. Using new materials ensures that there would be no chemical cross-contamination during the testing process. MSCPA representatives were responsible for applying the *Superstratum Hypochlorous Acid Pro Cleaner* according to the label directions.

A combination of four different mycotoxins produced by some of the most common indoor mold types were secured and mixed by MSCPA. Although it is unusual to find multiple types of mycotoxins on a single surface, this test approach represented a "worst-case" scenario to evaluate the efficacy of the Superstratum cleaner.

Specific Test Process

The actual testing involved intentional mycotoxin contamination of three, 4-inch square pieces of OSB. Mycotoxins used for the test included Gliotoxin, Trichothecenes (Group D), Ochratoxin A, and Zeralanone. The mycotoxin mixture was applied in a similar fashion to all three of the boards. Following the application of the mycotoxin mixture, and appropriate dwell time, the first board was sampled as a baseline. Sampling of the OSB contaminated material was done using an Environmental Mold and Mycotoxin Assessment (EMMA) swab from Real-Time Laboratory.

This test, using polymerase chain reaction (PCR) techniques for the analysis, confirmed and quantified the presence of all four types of mycotoxins on the inoculated OSB board.

The second OSB board, which also had been contaminated with mycotoxins, was sprayed with the *Hypochlorous Acid Pro Cleaner*. A hand sprayer with a coarse spray tip was used to apply the solution. The square was kept damp with the Superstratum solution by repeat spraying as necessary to keep it damp in conformance with the Superstratum instructions. After the manufacturer’s recommended 10-minute dwell time, the contaminated OSB was sampled with another swab which was analyzed by Real-Time Laboratory.

A third marked and inoculated OSB board was then sprayed with the Superstratum Cleaner; again using a hand sprayer with a coarse spray tip to apply the solution. On this board, regular agitation of the surface by wiping with a cotton rag dampened with the *Superstratum Hypochlorous Acid Pro Cleaner* was used to keep the square damp for the required 10 minutes. After the designated dwell/wiping time the square was sampled with an EMMA swab and analyzed to confirm the types and the quantity of mold and mycotoxins present by PCR analysis.

Results of the Testing

As detailed in the following chart, the results of the testing conducted by MSCPA in conjunction with Real-Time Laboratory showed an overall reduction in mycotoxins on the difficult to clean OSB boards of 94% - 95%.

The effectiveness of the *Superstratum Hypochlorous Acid Pro Cleaner* was relatively uniform for the different types of mycotoxins. Indeed, for three of the different types of mold related poisons that were tested, the cleaner removed 100% of the measurable mycotoxins. It was only the Trichothecenes type of mycotoxins where any residue was left behind after the spray or the spraying and wiping. For that mold related contaminant, spraying with the *Superstratum Hypochlorous Acid Pro Cleaner* removed 77% of the mycotoxins. When surface agitation through wiping was evaluated, the percentage of removed mycotoxins improved to 81%.

Superstratum Hypochlorous Acid Pro Cleaner Mycotoxin Removal Efficacy Test				
OSB board and treatment	Mycotoxins (parts per billion – ppb)			
	Gliotoxin	Trichothecenes	Ochratoxin A	Zearalenone
Contaminated control	5.057	0.891	8.275	1.292
Contaminated and sprayed	BMDL	0.205	BMDL	BMDL
Percent reduction	100%	77%	100%	100%
Contaminated and spray/wipe	BMDL	0.169	BMDL	BMDL
Percent reduction	100%	81%	100%	100%

BMDL = Below Method Detection Limit, or not present.

Addressing Mycotoxin Contamination Is A Process

Given the results of this testing, and the fact that reliable air samples which can identify mycotoxins are now available, Superstratum adjusted an existing protocol for conducting mold remediation. This revised remediation approach offers a process to their customers that is effective in addressing mycotoxins. While this process can be used for all mold remediation projects, it is critical that it be employed for situations where mold-sensitized occupants are present. Obviously, it should always be used for individuals who have been diagnosed with mycotoxins in their system.

Although the mycotoxin removal protocol utilizes the *Superstratum Hypochlorous Acid Pro Cleaner*, as one of the main components of the eradication of the poisons, it also incorporates additional steps. This “belt and suspenders” approach is justified by the significant health effects that can result from mycotoxin exposure and the recent research that confirms how difficult it is to remove the mold metabolites. Some redundancy is also required in order for the process to be in conformance with the current standard of care for the mold remediation industry²⁹.

Before the mycotoxin protocol begins, visible mold sources should be remediated with appropriate engineering controls in place, including but not limited to: isolation barriers, decontamination chamber, and HEPA filtered air mover to create negative pressure exhausted to the outdoors. Successful post remediation project testing should be conducted to confirm the effectiveness of mold source material.

Following the removal of mold source material, the Superstratum Mycotoxin Removal Technology (S.M.R.T.) Protocol can be utilized. That procedure includes:

1. Initial testing to confirm the presence of mycotoxins in the environment.
 - a. An initial assessment which incorporates both air and surface testing.
 - i. The testing should be detailed enough to identify and quantify the specific mycotoxins so that the information can be used as a pre-work baseline.
 - ii. The Superstratum recommended air test is the Respirare Labs Airborne Mycotoxin Environmental Assessment (AMEA) test.
2. Initial fog treatment of the entire structure with *Superstratum Hypochlorous Acid Cleaner*.
 - a. The chemical is safe to apply around people, pets, and plants.
 - b. Material is applied with foggers and sprayers
 - c. Because of their proximity to substantial quantities of the material in a short time period, applicators utilize appropriate personal protective equipment (PPE) during the application of the hypochlorous acid solution.
3. Detailed cleaning of all surfaces in the structure using *Superstratum Hypochlorous Acid Pro Cleaner*.

4. Second fogging of the structure with a diluted solution of *Superstratum Hypochlorous Acid Cleaner*
 - a. The fogging helps to reduce any remaining contaminants and provides additional humidity in the environment to improve the effectiveness of the gas phase treatment described in the next step.
 - b. Ideal humidity for chlorine dioxide (CLO₂) remediation bombs is 65%.
5. Gas phase treatment of the structure with chlorine dioxide.
 - a. Treatment follows the manufacturer's direction for the use of *Superstratum Remediation Bomb*.
 - b. Removal of people, pets, and plants for 12 hours while remediation bombs fill the structure with gaseous chlorine dioxide.
6. HEPA Filtered MCI (multi-cluster ionization) Positive Air Flush.
 - a. After roughly 12 hours of gas phase, the Superstratum team recommends airing out the structure with positive air pressure using HEPA Air Scrubber and downstream MCI Air Filtration device to push filtered and treated air into the home through controlled location(s) *i.e.*, through a window sealed and fitted with foam cutout or plastic and flex-duct.
7. Application of *Superstratum Smart Polymer Coating* to mold-prone surfaces in the structure.
 - a. Post application cleaning of residue from horizontal surfaces if necessary.
8. Post cleaning and treatment testing for mycotoxins to assure the effectiveness of the process.

Conclusion

The mold remediation industry continues to advance as both medical professionals and contractors see the strong connection between treating a person for illness mitigation and treating a structure that the person occupies for contamination control. As the understanding of the role mycotoxins play in the negative health outcomes of mold sensitized occupants grows, so does the emphasis on finding effective testing and remediation approaches. The recent advances in testing for mycotoxins in the air and on surfaces is now allowing mold remediation chemicals and work practices to be specifically evaluated for their effectiveness in addressing those naturally produced poisons. The Superstratum mycotoxin removal protocol utilizes a number of strategies for removing the hazardous mold byproducts along with the mold, including the proven results from applying their *Superstratum Hypochlorous Acid Pro Cleaner* product.

Michael Pinto, CSP, CMP, SMS, RTPE, FLS, ERS
CEO, Wonder Makers Environmental

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