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May 8, 2015
Client I.D. # 0423153MS
Re: Microbial Results Report from Cultured and Mycotoxins Sampling

Dear Mr. Robison:

This report is in regards to the microbial cultured sampling for mycotoxins analysis conducted at xxxxxxxx, Pennsylvania. The date of the sampling was Thursday April 23, 2015. Mark Stern, a Certified Indoor Environmentalist for NAL East, conducted the swab sampling in order to culture the sample and then it could be analyzed for mycotoxins.

The United States Environmental Protection Agency (US EPA) state the following information below about mold and mycotoxins. **Mold Toxins (Mycotoxins)**

Molds can produce toxic substances called mycotoxins. Some mycotoxins cling to the surface of mold spores; others may be found within spores. More than 200 mycotoxins have been identified from common molds, and many more remain to be identified. Some of the molds that are known to produce mycotoxins are commonly found in moisture-damaged buildings.

Exposure pathways for mycotoxins can include inhalation, ingestion, or skin contact. Although some mycotoxins are well known to affect humans and have been shown to be responsible for human health effects, for many mycotoxins, little information is available.

Many symptoms and human health effects attributed to inhalation of mycotoxins have been reported including: mucous membrane irritation, skin rash, nausea, immune system suppression, acute or chronic liver damage, acute or chronic central nervous system damage, endocrine effects, and cancer. More studies are needed to get a clear picture of the health effects related to most mycotoxins. However, it is clearly prudent to avoid exposure to molds and mycotoxins. Some molds can produce several toxins, and some molds produce mycotoxins only under certain environmental conditions. The presence of mold in a building does not necessarily mean that mycotoxins are present or that they are present in large quantities.

* Information on ingestion exposure, for both humans and animals, is more abundant–a wide range of health effects has been reported following ingestion of moldy foods including liver damage, nervous system damage and immunological effects. Much of the information on the human health effects of inhalation exposure to mycotoxins comes from studies done in the workplace and some case studies or case reports.*

Microbial Volatile Organic Compounds (mVOCs)

Some compounds produced by molds are volatile and are released directly into the air. These are known as microbial volatile organic compounds (mVOCs). Because these compounds often have strong and/or unpleasant odors, they can be the source of odors associated with molds. Exposure to mVOCs from molds has been linked to symptoms such as headaches, nasal irritation, dizziness, fatigue, and nausea. Research on mVOCs is still in the early phase.

The EPA states that as mold grows, it digests whatever it grows on. Unchecked mold growth can damage buildings and furnishings; molds can rot wood, damage drywall, and eventually cause structural damage to buildings. Mold can cause cosmetic damage, such as stains, to furnishings. The potential human health effects of mold are also a concern.

The goal of biological sampling is to help determine whether the biological particles present in a particular environment are affecting or causing irritation in certain individuals. Sampling is also used to locate the sources of indoor microorganisms and facilitate an effective remediation. While we are typically surrounded by a wide variety of different microorganisms every day, sampling provides us with a method to establish in a scientific way whether the environment in question contains more organisms than would normally be present.

Molds spores are found in virtually every property and can be detected, both indoors and outdoors, year round. Healthy indoor air is recognized as a basic right. People spend a large part of their time each day indoors: in homes, offices, schools, health care facilities, or other private or public buildings.

Problems of indoor air quality are recognized as important risk factors for human health in both low-income and middle- and high-income countries. Indoor air is also important because populations spend a substantial fraction of time within buildings. Exposure to microbial contaminants is clinically associated with respiratory symptoms, allergies, asthma and immunological reactions.

The **Centers for Disease Control and Prevention (CDC)** state that some people are sensitive to molds. For these people, exposure to molds can cause symptoms such as nasal stuffiness, eye irritation, wheezing, or skin irritation. Some people, such as those with serious allergies to molds, may have more severe reactions. Severe reactions may occur among workers exposed to large amounts of molds in occupational settings, such as farmers working around moldy hay. Severe reactions may include fever and shortness of breath. Some people with chronic lung illnesses, such as obstructive lung disease, may develop mold infections in their lungs.

The **Environmental Protection Agency (EPA)** state that when mold spores land on a damp spot, they may begin growing and digesting whatever they are growing on in order to survive. Since molds gradually destroy the things they grow on, you should try to prevent damage to building materials and furnishings by eliminating mold growth.

When moisture problems occur and mold growth results, building occupants may begin to report odors and a variety of health problems, such as headaches, breathing difficulties, skin irritation, allergic reactions, and aggravation of asthma symptoms; all of these symptoms could potentially be associated with mold exposure. All molds have the potential to cause health effects. Molds produce allergens, irritants, and in some cases, toxins that may cause reactions in humans.

In **2004 the Institute of Medicine (IOM)** found there was sufficient evidence to link indoor exposure to mold with upper respiratory tract symptoms, cough, and wheeze in otherwise healthy people; with asthma symptoms in people with asthma; and with hypersensitivity pneumonitis in individuals susceptible to that immune-mediated condition. The IOM also found limited or suggestive evidence linking indoor mold exposure and respiratory illness in otherwise healthy children.

In **2009, the World Health Organization** issued additional guidance, the WHO Guidelines for Indoor Air Quality: Dampness and Mold. Other recent studies have suggested a potential link of early mold exposure to development of asthma in some children, particularly among children who may be genetically susceptible to asthma development, and that selected interventions that improve housing conditions can reduce morbidity from asthma and respiratory allergies, but more research is needed in this regard.

NAL East conducted a total of two (2) direct examination swab samples.

1. Basement By HVAC Unit (swab)
2. Basement By Sump-Pump (swab)

A **direct examination** allows for the determination of the presence of fungal spores as well as what types of fungi are present. Direct sampling indicates all molds present in a given area and may reveal indoor reservoirs of spores that have not yet become airborne. Direct exam sampling is a non-destructive technique that allows for the determination of possible microbial contamination of suspect sites such as visibly stained or discolored areas.

Once the samples were collected, they were sealed, labeled, put in a Ziploc bag, double bagged and sent to an independent laboratory.

Once the direct examination sample was taken, it was cultured. **The objectives of culturable sampling** is to capture and quantify the different culturable fungal spores present to determine whether the levels present indicate a fungal problem in the indoor locations. Culturable sampling allows for the differentiation of *Aspergillus* and *Penicillium*, standard speciation of *Aspergillus* and speciation of other fungi when required. It also provides counts indicative of how many spores are culturable and present. It assesses the viability of many fungi. After incubation, colonies of each genus can then be counted and identified. This can be critical in certain situations when severely immunocompromised people are present.

The results from the cultured swab sample that was conducted in the **Basement by the HVAC Unit (sample #2)** indicate the presence of three (3) different species, one (1) of them being Paecilomyces sp. (4,000 CFU/Swab). Paecilomyces sp. is linked to Type 1 Allergies and Type 3 Hypersensitivity. The total fungi count in this sample was 4,300 CFUs (CFU/Swab). A swab sample is an indication of what is present, only in the area sampled (a couple of inches).

The results from the cultured swab sample that was conducted in the **Basement by the Sump-Pump (sample #3)** indicate the presence of nine (9) different species, one (1) of them being Paecilomyces sp. (4,000 CFU/Swab). Paecilomyces sp. is linked to Type 1 Allergies and Type 3 Hypersensitivity. The total fungi count in this sample was 4,300 CFUs (CFU/Swab). A swab sample is an indication of what is present, only in the area sampled (a couple of inches).

After the samples were cultured, it was decided that the best two mycotoxins group to sample for was Sterigmatocystin **(sample #1),** Basement By HVAC Unit and Ochratoxin A **(sample #2)**, Basement By Sump-Pump. Sample #1, sampling for Sterigmatocystin, was much higher (160 ug/swab) than the detection limit (0,010 ug/swab) and deemed elevated. The Ochratoxin sample results (sample #2) were low. Intense cleanup is highly recommended based on what was detected in sample #1.

Sterigmatocystin is a toxic metabolite structurally closely related to the aflatoxins and consists of a xanthone nucleus attached to a bifuran structure. Sterigmatocystin is mainly produced by the fungi Aspergillus nidulans and A. versicolor. It has been reported in moldy grain, green coffee beans and cheese although information on its occurrence in foods is limited. It appears to occur much less frequently than the aflatoxins, although analytical methods for its determination have not been as sensitive until recently, and so it is possible that small concentrations in food commodities may not always have been detected. Although it is a potent liver carcinogen similar to aflatoxin B1, current knowledge suggests that it is nowhere near as widespread in its occurrence.

The toxic effects of sterigmatocystin are much the same as those of aflatoxin B1. It is thus considered as a potent carcinogen, mutagen, and teratogen. It is less acutely toxic to rodents and monkeys but appears to be slightly more toxic to zebra fish. Toxic effects of sterigmatocystin-fed laboratory animals have included kidney and liver damage and diarrhoea. Skin and hepatic tumours are induced in rats by dermal application. Cattle exhibiting bloody diarrhoea, loss of milk production and in some cases death were found to have ingested feed containing Aspergillus versicolor and high levels of sterigmatocystin of about 8 mg/kg. The acute toxicity, carcinogenicity, and metabolism of sterigmatocystin has been compared with those for aflatoxin and several other hepatotoxic mycotoxins (Wikipedia).

The IARC-classification of sterigmatocystin is group 2B, which means it is carcinogenic in other species and is possibly carcinogenic to humans, but that a definitive link between human exposure and cancer has not been proven.

A **mycotoxin** (from Greek μύκης (mykes, mukos) "fungus" and τοξικόν (toxikon) "poison")[1][2] is a toxic secondary metabolite produced by organisms of the fungi kingdom, commonly known as molds.[3] The term 'mycotoxin' is usually reserved for the toxic chemical products produced by fungi that readily colonize crops.[4] One mold species may produce many different mycotoxins, and the same mycotoxin may be produced by several species.[5]

Aflatoxins are a type of mycotoxin produced by *Aspergillus* species of fungi, such as *A. flavus* and *A. parasiticus*.[18] The umbrella term aflatoxin refers to four different types of mycotoxins produced, which are B_1, B_2, G_1, and G_2.[19] Aflatoxin B_1, the most toxic, is a potent carcinogen and has been directly correlated to adverse health effects, such as liver cancer, in many animal species.[18] Aflatoxins are largely associated with commodities produced in the tropics and subtropics, such as cotton, peanuts, spices, pistachios and maize.[18][19]

Citrinin is a toxin that was first isolated from *Penicillium citrinum*, but has been identified in over a dozen species of *Penicillium* and several species of *Aspergillus*. Some of these species are used to produce human foodstuffs such as cheese (*Penicillium camemberti*), sake, miso, and soy sauce (*Aspergillus oryzae*). Citrinin is associated with yellowed rice disease in Japan and acts as a nephrotoxin in all animal species tested.[22] Although it is associated with many human foods (wheat, rice, corn, barley, oats, rye, and food colored with Monascus pigment) its full significance for human health is unknown. Citrinin can also act synergistically with Ochratoxin A to depress RNA synthesis in murine kidneys.[11]

Ergot Alkaloids are compounds produced as a toxic mixture of alkaloids in the sclerotia of species of *Claviceps*, which are common pathogens of various grass species. The ingestion of ergot sclerotia from infected cereals, commonly in the form of bread produced from contaminated flour, cause ergotism the human disease historically known as St. Anthony's Fire. There are two forms of ergotism: gangrenous, affecting blood supply to extremities, and convulsive, affecting the central nervous system. Modern methods of grain cleaning have significantly reduced ergotism as a human disease, however it is still an important veterinary problem. Ergot alkaloids have been used pharmaceutically.[11]

Fusarium toxins are produced by over 50 species of *Fusarium* and have a history of infecting the grain of developing cereals such as wheat and maize.[25][26] They include a range of mycotoxins, such as: the **fumonisins**, which affect the nervous systems of horses and may cause cancer in rodents; the **trichothecenes**, which are most strongly associated with chronic and fatal toxic effects in animals and humans; and **zearalenone**, which is not correlated to any fatal toxic effects in animals or humans. Some of the other major types of *Fusarium* toxins include: beauvercin and enniatins, butenolide, equisetin, and fusarins.[27]

Ochratoxin is a mycotoxin that comes in three secondary metabolite forms, A, B, and C. All are produced by *Penicillium* and *Aspergillus* species.

The three forms differ in that Ochratoxin B (OTB) is a nonchlorinated form of Ochratoxin A (OTA) and that Ochratoxin C (OTC) is an ethyl ester form Ochratoxin A.[20] *Aspergillus ochraceus* is found as a contaminant of a wide range of commodities including beverages such as beer and wine. *Aspergillus carbonarius* is the main species found on vine fruit, which releases its toxin during the juice making process.[21] OTA has been labeled as a carcinogen and a nephrotoxin, and has been linked to tumors in the human urinary tract, although research in humans is limited by confounding factors.[20][21]

Patulin is a toxin produced by the *P. expansum*, *Aspergillus*, *Penicillium*, and *Paecilomyces* fungal species. *P. expansum* is especially associated with a range of moldy fruits and vegetables, in particular rotting apples and figs.[23][24] It is destroyed by the fermentation process and so is not found in apple beverages, such as cider. Although patulin has not been shown to be carcinogenic, it has been reported to damage the immune system in animals.[23] In 2004, the European Community set limits to the concentrations of patulin in food products. They currently stand at 50 μg/kg in all fruit juice concentrations, at 25 μg/kg in solid apple products used for direct consumption, and at 10 μg/kg for children's apple products, including apple juice.[23][24]

Trichothecenes are a very large family of chemically related mycotoxins produced by various species of *Fusarium*, *Myrothecium*, *Trichoderma*, *Trichothecium*, *Cephalosporium*, *Verticimonosporium*, and *Stachybotrys*. Trichothecenes belong to sesquiterpene compounds. The most important structural features causing the biological activities of trichothecenes are: the 12,13-epoxy ring, the presence of hydroxyl or acetyl groups at appropriate positions on the trichothecene nucleus and the structure and position of the side-chain. They are produced on many different grains like wheat, oats or maize by various *Fusarium* species such as *F. graminearum*, *F. sporotrichioides*, *F. poae* and *F. equiseti*.

Some molds that produce trichothecene mycotoxins, such as *Stachybotrys chartarum*, can grow in damp indoor environments. It has been found that macrocyclic trichothecenes produced by *Stachybotrys chartarum* can become airborne and thus contribute to health problems among building occupants.[1][2]

Please read all information at the bottom of the lab charts for further clarification.

Conclusion

Based on your sample results, intense cleaning should be conducted in the areas sampled. A swab sample is an indication of what is present in a specific area. Paecilomyces can produce mycotoxins and it should not be present in a remediated area. In addition, these sample results do not mean that no Stachybotrys was present, it just wasn't detected in the areas sampled.

> *Many fungi (e.g., species of* Aspergillus, Penicillium, Fusarium, Trichoderma, *and* Memnoniella*) in addition to SC can produce potent mycotoxins, some of which are identical to compounds produced by SC.*

Mycotoxins are fungal metabolites that have been identified as toxic agents. For this reason, SC cannot be treated as uniquely toxic in indoor environments.

People performing renovations/cleaning of widespread fungal contamination may be at risk for developing Organic Dust Toxic Syndrome (ODTS) or Hypersensitivity Pneumonitis (HP). ODTS may occur after a single heavy *exposure to dust contaminated with fungi and produces flu-like symptoms. It differs from HP in that it is not an immune-mediated disease and does not require repeated exposures to the same causative agent. A variety of biological agents may cause ODTS including common species of fungi. HP may occur after repeated exposures to an allergen and can result in permanent lung damage.*

Fungi can cause allergic reactions. The most common symptoms are runny nose, eye irritation, cough, congestion, and aggravation of asthma.

There are two (2) industry-accepted reference guidelines that we are confident in. It is best to use these guidelines to ensure the effectiveness of the remediation work being conducted. The first is the ***New York City Department of Health Guidelines on Assessment and Remediation of Fungi in Indoor Environments*** and the second one is the ***Institute of Inspection, Cleaning and Restoration's (IICRC) S520 Standard and Reference Guide for Professional Mold Remediation.***

Use those guidelines if you are unsure about any remediation protocols. This document is not a legal mandate and should be used as a guideline. Currently there are no United States Federal, New York State, or New York City regulations for evaluating potential health effects of fungal contamination and remediation. These guidelines are subject to change as more information regarding fungal contaminants becomes available.

Adverse health affects in affected individuals can include both illnesses and allergic responses.

Some people are sensitive to molds. For these people, exposure to molds can cause symptoms such as nasal stuffiness, eye irritation, wheezing, or skin irritation.
Some people, such as those with serious allergies to molds, may have more severe reactions. Severe reactions may occur among workers exposed to large amounts of molds in occupational settings, such as farmers working around moldy hay. Severe reactions may include fever and shortness of breath. Some people with chronic lung illnesses, such as obstructive lung disease, may develop mold infections in their lungs.

It was mentioned that you have an apparatus that helps with the reduction of mycotoxins. It is extremely important in conducting the proper clean-up to help reduce health concerns inside the property.

NAL East currently follows the guidelines set forth by the United States Environmental Protection Agency (US EPA), the New York City Department of Health (NYCDOH) and the California Occupational Health and Safety Administration (Cal-OSHA).

If you have any questions regarding this report, please feel free to contact us anytime.

Sincerely,

Mark Stern
Council-Certified Indoor Environmentalist (CIE # 01444)
Council-Certified Microbial Remediator (CMR # 03681)
Council-Certified Microbial Remediation Supervisor (CMRS # 0704024)
EPA Certified AHERA Asbestos Building Inspector (PA # 045363)
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June 10, 2015
Client I.D. # 0522155MS
Re: Mycotoxins Sampling Results Report

Dear Mr. Robison:

This report is in regards to the microbial cultured sampling for mycotoxins analysis conducted at xxxxxx, Pennsylvania. The date of the sampling was Friday May 22, 2015. Mark Stern, a Certified Indoor Environmentalist for NAL East, conducted the swab sampling which was delivered to the laboratory to be analyzed for mycotoxins.

The United States Environmental Protection Agency (US EPA) state the following information below about mold and mycotoxins. **Mold Toxins (Mycotoxins)**

Molds can produce toxic substances called mycotoxins. Some mycotoxins cling to the surface of mold spores; others may be found within spores. More than 200 mycotoxins have been identified from common molds, and many more remain to be identified. Some of the molds that are known to produce mycotoxins are commonly found in moisture-damaged buildings.

Exposure pathways for mycotoxins can include inhalation, ingestion, or skin contact. Although some mycotoxins are well known to affect humans and have been shown to be responsible for human health effects, for many mycotoxins, little information is available.

Many symptoms and human health effects attributed to inhalation of mycotoxins have been reported including: mucous membrane irritation, skin rash, nausea, immune system suppression, acute or chronic liver damage, acute or chronic central nervous system damage, endocrine effects, and cancer. More studies are needed to get a clear picture of the health effects related to most mycotoxins. However, it is clearly prudent to avoid exposure to molds and mycotoxins. Some molds can produce several toxins, and some molds produce mycotoxins only under certain environmental conditions. The presence of mold in a building does not necessarily mean that mycotoxins are present or that they are present in large quantities.

* Information on ingestion exposure, for both humans and animals, is more abundant–a wide range of health effects has been reported following ingestion of moldy foods including liver damage, nervous system damage and immunological effects. Much of the information on the human health effects of inhalation exposure to mycotoxins comes from studies done in the workplace and some case studies or case reports.*

Microbial Volatile Organic Compounds (mVOCs)

Some compounds produced by molds are volatile and are released directly into the air. These are known as microbial volatile organic compounds (mVOCs). Because these compounds often have strong and/or unpleasant odors, they can be the source of odors associated with molds. Exposure to mVOCs from molds has been linked to symptoms such as headaches, nasal irritation, dizziness, fatigue, and nausea. Research on mVOCs is still in the early phase.

NAL East conducted a total of two (2) direct examination swab samples to determine the presence of mycotoxins.

1. Basement By HVAC Unit (swab)
2. Basement By HVAC Unit (swab)

A **direct examination** allows for the determination of the presence of fungal spores as well as what types of fungi are present. Direct sampling indicates all molds present in a given area and may reveal indoor reservoirs of spores that have not yet become airborne. Direct exam sampling is a non-destructive technique that allows for the determination of possible microbial contamination of suspect sites such as visibly stained or discolored areas.

Once the samples were collected, they were sealed, labeled, put in a Ziploc bag, double bagged and sent to EMSL Analytical in Cinnaminson New Jersey for the analysis of mycotoxins.

The results from the first swab sample that was conducted in the **Basement on the HVAC Unit (sample #1)** indicates that the Ochratoxin A Compound, Mycotoxin was detected at less than the reporting limit (<0.010), and deemed acceptable. A swab sample is an indication of what is present in the sampled area.

The results from the second swab sample that was conducted in the **Basement on the HVAC Unit (sample #2)** indicates that Sterigmatocystin Mycotoxins was detected at less than the reporting limit (<0.010), which is acceptable as well. In the initial sampling, the Sterigmatocystin was much higher (160 ug/swab) than the detection limit of (0,010 ug/swab) and deemed elevated.

Conclusion

Based on your sample results, nothing was detected over the detection limit. Whatever machinery that was installed in the property appears to have eliminated what was present before the equipment was placed in the property. If the HVAC unit wasn't wiped down, which it appears that it wasn't, the machinery must have worked.

It was mentioned that an apparatus (piece of machinery) that helps with the reduction of mycotoxins was placed in the property. The levels present before the machinery was in operation appear to have been significantly reduced, not detectable.

It appears that whatever was detected in the initial sampling, was not detected or present now.

Please read all information at the bottom of the lab charts for further clarification.

NAL East currently follows the guidelines set forth by the United States Environmental Protection Agency (US EPA), the New York City Department of Health (NYCDOH) and the California Occupational Health and Safety Administration (Cal-OSHA).

If you have any questions regarding this report, please feel free to contact us anytime.

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