

## COMMENTS ON THE EXPOSURE OF OCCUPANTS TO MYCOTOXINS IN THE INDOOR ENVIRONMENT

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### MYCOTOXINS

Mycotoxins (toxins produced by fungi) are relatively low molecular weight, nonvolatile chemicals. They are secondary metabolites, and therefore the fungus must expend energy to produce them. This factor limits their production unless a fungal colony is under stress; then they are typically produced as a defense against competing fungi. Therefore, not all colonies of a toxigenic fungus may produce mycotoxins; and those colonies that do produce them may exhibit a wide variation in concentration and/or potency.

Mycotoxins are produced by a wide range of fungal species [Koppen], but not all fungal species produce potent mycotoxins that may be expected to affect human health. Those that do are often referred to as “toxigenic” fungi [toxigenic: capable of producing mycotoxins]. Toxigenic fungi not only include contaminant fungi (*Aspergillus*, *Penicillium*, *Fusarium*, *Stachybotrys*), but several fungi considered to be common environmental fungi (*Alternaria*, *Rhizopus*) have also been implicated in mycotoxicosis (toxicity resulting from absorbing a toxic dose of fungal toxins) [Ostry, Vallabhaneni]. In addition, a specific mycotoxin may only be produced by a limited number of fungi. For example, aflatoxin B1 is produced by *Aspergillus* species such as *A. flavus* and *A. parasiticus*, but it is not produced by *Stachybotrys* or *Fusarium* (which produce tricothecenes and other mycotoxins).

These natural variations in the immediate environment of a fungal colony can result in (1) a wide range in the amount and potency of mycotoxin in colonies of the same fungal species, (2) highly variable results if only one of many colonies are sampled, and (3) different occupant responses when the same toxigenic fungus is detected in different indoor environments [Bush]. These factors often make it difficult to (1) collect representative samples from the indoor environment, and (2) associate adverse health effects with the results of environmental sampling.

### SOURCES OF MYCOTOXIN EXPOSURE

Inhalation of mycotoxins has been shown to be a significant issue in industrial settings (farm workers, swine and cowsheds, grain processing plants, wineries, etc.) [Breraa, Skaug]. Mycotoxins have also been proposed as the cause of adverse health effects from the inhalation of airborne mold in residential, school, and office environments. However, evidence has been inadequate to establish a causal relationship between the inhalation of indoor mold and health effects related to mycotoxin exposure [Kelman]. Therefore, except in industrial settings, inhalation of mycotoxins is not expected to be an important route of exposure.

Occupant exposures typically result from the ingestion of food items such as grains, meats, dairy products, nuts, fruits, etc. [Koppen, Lopez, Ostry]. Mycotoxins are naturally occurring contaminants in the food chain, with the following five types of mycotoxins especially important in human health:

aflatoxins, ochratoxin A, fumonisins, trichothecenes, and zearalenone [Pitta]. The most important mycotoxins in the food chain are: (1) the aflatoxins produced by *Aspergillus* species, (2) ochratoxin A and patulin produced by *Aspergillus* and *Penicillium* species, and (3) mycotoxins from the genus *Fusarium* [Krska]. Some of the most susceptible foodstuffs to aflatoxins and ochratoxin A are cocoa, coffee and spices [Breraa]. It is also interesting to note that some dietary supplements, sometimes prescribed to treat mycotoxicosis, have also been confirmed as a potential source of mycotoxin exposure [Vallabhaneni].

Mycotoxins may be present in a wide variety of foods: grain products, dairy products, nuts and legumes, tomato products, beer and wine, chocolate, dried grapes, farmed fish, shellfish, and meats [NGFA]. They are resistant to heat, and survive temperatures typically used to prepare foods [Pitta]. However, the amounts of these mycotoxins in grains, animal feed, and foods are regulated in the US and many other countries. Therefore, although dietary exposures are expected to be quite low, the ingestion of mycotoxins may be expected to be both common and frequent.

Since low levels of mycotoxins are found in many foods, many sensitized individuals modify their diets in an effort to avoid exposure to the extent possible (for example, avoiding grain products to minimize tricothecene exposure). However, this can be a daunting task. Low levels of mycotoxins are present in a wide range of food items; and are so common as to require regulatory limits to protect the public. As a result of this wide-spread presence in our foods, mycotoxins may be detected in the urine of even healthy persons [Kawamoto].

#### **HEALTH EFFECTS OF MYCOTOXINS**

A mycotoxicosis is not an infection; and it should be noted that antifungal medications that are used to treat fungal infections are not effective for treating illnesses caused by mycotoxins [Kawamoto]. Mycotoxicosis also differs from immune responses such as the inflammation induced by IgE or IgG antibodies. Allergic (IgE) or hypersensitivity (IgG) reactions of sensitized individuals may be triggered by a relatively low "threshold" exposure; whereas a toxic response is typically proportional to the dose (amount of mycotoxin absorbed by the individual).

For an individual to experience a toxic effect: (1) the toxin must be present in their environment, (2) there must be sufficient mycotoxin present so that absorption of a toxic dose is possible, (3) there must be a route of exposure [inhalation, ingestion] that allows the mycotoxin to be absorbed, and (4) the occupant must absorb a sufficient dose for the mycotoxin to have a toxic effect [Bush].

A patient's symptoms should be evaluated by a physician in order to arrive at a diagnosis of mycotoxicosis. Individual mycotoxins can be genotoxic, mutagenic, carcinogenic, teratogenic, estrogenic, cytotoxic, or immunosuppressive [Koppen, Krska]. These are the diseases commonly associated with mycotoxicosis.

One limitation faced by physicians is that little information is available on the levels of mycotoxins in body fluids and biological tissues that are associated with disease. One reason is that routine methods of analysis may not be available for all mycotoxins. Many of these methods are considered to be "research methods".

A second limitation is a biological mechanism referred to as “toxicokinetics”. Whenever a drug or toxin enters the body, it immediately begins to be detoxified. That’s why we have to take prescribed medicines, regularly and repeatedly, to maintain their effectiveness. Testing patients for the presence of mycotoxins is complicated by the fact that mycotoxins are not cumulative toxins [Sudakin]. Unless exposure is continuous, the concentration of mycotoxin in body fluids begins to decrease as soon as the exposure stops. However, very little information is available relating to the persistence of mycotoxins in humans or their body fluids [Sudakin].

The time it takes the body to detoxify half of the concentration of the toxin is the “half-life”. It should be noted that the detection of a mycotoxin or its metabolites in urine or feces is an indication that the body is processing the toxin. Mycotoxins have half-lives (a measure of persistence) ranging from hours to days, depending on the specific mycotoxin [Bush]. For example, the half-life of aflatoxin B1 is about 92 hours, while the half-life of DON (vomitoxin) is about 7 hours [Rosen] [*After 92 hours (3.8 days), the concentration of aflatoxin B1 would be 50% of the original concentration; 25% after 7.6 days; and 12.5% after 11.4 days.*].

Therefore, when biological fluids are collected for testing relative to the last exposure may be an important parameter (When was the patient’s sample collected relative to the date of their last exposure?). This factor complicates the interpretation of mycotoxin concentrations in urine or other body fluids. A second issue is the lack of approved, validated laboratory methods for measuring the amount of mycotoxin in body fluids and biological tissues on a routine basis. Laboratories often have a Clinical Laboratory Improvement Amendments (CLIA) certification, which addresses the quality control and procedural standards used by the laboratory to ensure accurate and reliable test results. However, CLIA does not address the clinical validity of the test results (i.e., the accuracy with which the test identifies, measures, or predicts a patient’s clinical status). There is currently no FDA-approved test for mycotoxins in human urine; and urine mycotoxin tests are not approved by the FDA for accuracy or for clinical use. [Kawamoto].

## **DETECTING MYCOTOXINS**

### **Assessing Occupant Exposure**

Presumptive evidence that an individual has been exposed to mycotoxins may be based on a diagnosis of patient symptoms by a physician, or by the detection of mycotoxins in their environment. Sampling an indoor environment for mycotoxins can be a difficult task, often producing results with low reliability. It may be especially difficult to collect representative samples in an indoor environment with a limited budget.

One of the primary challenges of assessing mycotoxin exposures is the collection of representative samples, with the sampling step typically contributing the largest variability in mycotoxin determinations [Koppen, Krska]. This makes it difficult to reliably detect mycotoxins in both the food chain and the indoor environment. For example, submitting a nonrepresentative, highly variable sample for analysis by a sophisticated laboratory method will result in a nonrepresentative, highly variable sample result. *Aspergillus* species, especially, are prone to producing localized areas of mycotoxin within a larger sample matrix.

Second, mycotoxins are nonvolatile; and, if found in the respirable air, are typically associated with solid particles such as mold spores, hyphal fragments, or micro-particles. In order for the mycotoxin to be

absorbed by the occupant, the contaminated surface dust must be inhaled; and, in sufficient amounts for a toxic dose to be absorbed. Therefore, the occurrence of mycotoxins in surface dust samples should not be interpreted as an indicator of occupant exposure [Bush].

Third, to make sampling for mycotoxins even more complicated, the collection of short-term (less than 60 minute) airborne samples is not recommended because of the low concentrations typically present in indoor spaces. Longer sampling times (8-12 hours) may be needed to collect sufficient mycotoxin to be detected by the laboratory. The conclusion is that sampling for mycotoxins in the indoor environment, either surface samples or airborne samples, may be of limited value for assessing occupant exposure.

### **Sample Collection**

However, sampling may be requested by the occupant, or deemed to be necessary by the inspector. One method for collecting a more representative sample, while controlling the cost, is to collect composite samples.

Composite Sample: Use one sampler to collect a sample from multiple areas within the indoor space. The objective is to obtain an “average” result for the entire space. For example, use a filter cassette to collect a micro-vacuum sample from the carpet, the couch, the top of a door jam, the air return filter, etc. The sample is a “composite” of the indoor dust; and the sample result is an average of the occupant exposure potential. A composite sample provides four benefits: (1) it limits the cost of sampling, (2) it reduces variability, (3) it increases the amount of sample available for analysis, and (4) the average concentration is the parameter most closely associated with occupant dose.

There are two additional issues that substantially affect whether or not the sample result is representative of the indoor space: (1) how the sample is collected, and (2) how it is analyzed by the laboratory [Spurgeon]. These factors are actually discussed in reverse order to make the reasoning more apparent.

### **Laboratory Analysis**

The sample received by the laboratory may contain a large quantity of dust, much more than is needed for analysis. The laboratory will typically retain only a small portion of the dust for analysis, discarding most of the sample. This greatly reduces the effectiveness of the “averaging” process, and re-introduces greater variability.

In order to have maximum value to the occupant and/or their physician, the sample result should reflect the occupant’s environment – it should be representative of that environment. Typically, a large amount of dust may be collected by the inspector. As an example, let’s assume 1,000 milligrams of dust were collected and submitted to a laboratory. Most laboratories will select a 5 milligram portion of the dust for analysis, discarding the remaining 995 milligrams. In this example, the potential for adverse health effects would be assessed using 0.5% of the sample, while 99.5% of the sampled dust was discarded. The concern: how well does the 5 mg of dust retained by the laboratory represent the mycotoxins in the 1,000 milligram dust sample that was collected? Minimizing variability means (1) the laboratory must mix the original sample very well prior to selecting the 5 mg portion for analysis, or

(2) a sampling method should be used that only collects a small amount of dust so very little has to be discarded.

## Sample Collection Methods

The need to obtain a representative sample result influences the selection of the sampling method. The preferred sampling method should (1) be suitable for laboratory analysis, (2) be usable for collecting composite samples, (3) collect the minimum amount of dust necessary to be representative of the indoor space, and (4) collect dust from both the surface and interior of the item sampled. These requirements are satisfied by using micro-vacuum filter cassettes to collect dust samples.

First, the filter media in a cassette is suitable for laboratory analysis. Filter cassettes are a common sampling method; and filters are commonly analyzed by microscopy, culturing, and/or qPCR.

Second, a filter cassette is capable of collecting a composite sample from multiple surfaces. For example, one cassette could be used to sample carpet dust, couch cushions, settled dust on door jambs, and then the dust collected on an air return filter. This is more difficult to do with a swab, since it is difficult to retain previously collected dust on the swab.

Third, the open-face fixed area (OFFA) method collects a minimal weight of dust, but has been shown to be a reproducible sampling method [Spurgeon]. The laboratory is typically instructed to analyze the entire filter when this method is used. Therefore, instead of assessing occupant exposure potentials using 0.5% of the sample, 100% of the sample is used. Therefore, the reported sample results are both more representative of the space sampled, and more reproducible.

Fourth, filter cassettes use a vacuum to collect the dust. This draws contaminants both from the surface and deep within the item being sampled. For example, compare tape or swab sampling with cassette sampling for carpets and couch cushions. A tape lift or swab only collects contaminants from the tops of carpet fibers, or from the surface of a couch cushion. In comparison, a filter cassette vacuums contaminants from the carpet fibers, the dust embedded in the carpet mat, and from the pad beneath the carpet. If sampling a couch cushion, a vacuum cassette draws contaminants from the surface fabric as well as the interior of the foam cushion. Therefore, entirely different results may be obtained for tape/swab samples and micro-vacuum samples.

## Wikipedia

### Health Effects of Mycotoxins

Genotoxic: genotoxicity describes the property of chemical agents that damages the genetic information within a cell causing mutations, which may lead to cancer. While genotoxicity is often confused with mutagenicity, all mutagens are genotoxic, whereas not all genotoxic substances are mutagenic.

Mutagenic: a mutagen is a physical or chemical agent that changes the genetic material, usually DNA, of an organism and thus increases the frequency of mutations above the natural background level. As many mutations can cause cancer, mutagens are therefore also likely to be carcinogens.

Carcinogenic: Any substance that is an agent directly involved in causing cancer. There are many natural carcinogens. Aflatoxin B1, which is produced by the fungus *Aspergillus flavus* growing on stored grains, nuts and peanut butter, is an example of a potent, naturally occurring microbial carcinogen.

Teratogenic: An agent causing abnormalities of physiological development; including human congenital abnormalities, but taking into account other developmental stages including puberty.

Estrogenic: Any substance, natural or synthetic, that mimics the effects of the natural hormone estrogen; the primary female sex hormone. It is responsible for the development and regulation of the female reproductive system and secondary sex characteristics.

Cytotoxic: cytotoxicity is the quality of being toxic to cells. Exposing cells to the cytotoxic compound can result in a variety of cell fates, including necrosis (death) of the cell.

Immunosuppressive: immunosuppression is a reduction of the activation or efficacy of the immune system. An immunosuppressant is any agent that weakens the immune system, including immunosuppressive drugs and some environmental toxins.

## Major Groups of Mycotoxins

Aflatoxins are a type of mycotoxin produced by *Aspergillus* species of fungi, such as *A. flavus* and *A. parasiticus*. The umbrella term aflatoxin refers to four different types of mycotoxins produced, which are B1, B2, G1, and G2. Aflatoxin B1, the most toxic, is a potent carcinogen and has been directly correlated to adverse health effects, such as liver cancer, in many animal species. Aflatoxins are largely associated with commodities produced in the tropics and subtropics, such as cotton, peanuts, spices, pistachios, and maize.

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. 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.

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; causes 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.

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. 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.

Gliotoxin is produced by several species of fungi, especially those of marine origin. It is produced by human pathogens such as *Aspergillus fumigatus*, and also by species of *Trichoderma*, and *Penicillium*. Gliotoxin possesses immunosuppressive properties.

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. *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. 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.

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. 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. 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.

Tricothecenes may be produced by fungi such as *Acremonium*, *Fusarium* species, *Stachybotrys*, and *Trichoderma*.

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