MYCOTOXINS

CHARACTERISTICS, SAMPLING METHODS, AND LIMITATIONS

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ENVIROCHECK, INC.
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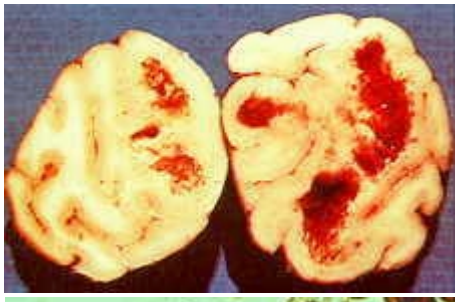
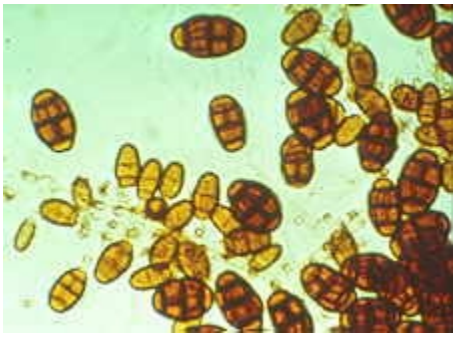


Mycotoxins - "hole-in-the-head disease" of horses - leucoencephalomalacia, caused by toxins of *Fusarium moniliforme.*.



Mycotoxins - *Pithomyces chartarum,* which causes facial eczema of sheep.

(Kendrick, 2002)

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Definitions and Health Effects

Some molds produce mycotoxins: low-molecular weight fungal secondary metabolites that are capable of initiating a toxic response in vertebrates (Adams, 2002; Davis, 2001). Mycotoxins are considered secondary metabolites because they are not essential for maintaining mold cells or fungal growth, but are derived from primary metabolic process chemicals such as polypeptides, amino acids, phenols, or terpenoids (Microbial Diagnosis, 2002). Mycotoxins are believed to provide competitive advantages for fungi such as assisting parasitic fungi with invading host tissue, or helping to eliminate other competing organisms in the same environment (Adams, 2002). "Higher organisms are not specifically targeted by mycotoxins, but seem to be caught in the crossfire of the biochemical warfare among mold species…and bacteria vying for the same ecological niche…(including some found indoors in contaminated buildings)" (Mold-Help, 2001).

MVOCs (microbial volatile organic compounds) differ from mycotoxins in that mVOCs are readily separated from spores and vegetative cells, and exist as gases at room temperature. Mycotoxins are generally not volatile, and are primarily associated with spores (Nielsen, 2002; Mold-Help, 2001). Both mycotoxins and mVOCs have been shown capable of causing short term and long-term health effects (Microbial Diagnosis, 2002).

Nearly all mycotoxins are cytotoxic, disrupting various cellular structures such as membranes, and interfering with vital processes such as protein, RNA and DNA synthesis (Mold-Help, 2001). "Molds known to potentially produce mycotoxins and which have been isolated in infestations causing adverse health effects include certain

species of *Acremonium, Alternaria, Aspergillus, Chaetomium, Cladosporium, Fusarium, Paecilomyces, Penicillium, Stachybotrys, and Trichderma*" (Davis, 2001). Until recently, human health effect studies for mycotoxin exposure have generally prevailed on the topic of ingestion. Consumption of mycotoxins has shown immunosuppression, mutagenicity, and cancer as well as adverse effects on various organs and systems of the body including kidney and liver, gastrointestinal, nervous, urogenital, and vascular (FAO/IAEA, 1997). Additional symptoms of mycotoxins exposure can include dermatitis, cold and flu symptoms, sore throat, headache, fatigue, and diarrhea (Mold-Help, 2001). Only a very limited number of studies have been conducted on the role of mycotoxins in indoor air and human health, therefore the role of mycotoxins remains controversial (Miller, et al, 2001). In a 2002 case study, Johanning, et al. found that,

> "highly toxic air filter results from our case investigations clearly support the hypothesis of airborne mycotoxins exposure and related inhalation risk to patients in indoor environments with known toxigenic fungi."

A single type of mycotoxin can often cause more than one type of toxic effect, and the production of mycotoxins is not solely dependant on the type or toxicity of a particular mold strain (Adams, 2002). Specific environmental conditions are believed needed for fungi to produce mycotoxins, such as the composition of the substrate, water activity, pH-value, temperature, oxygen, and the presence of competitive organisms (Johanning, et al 2002; Davis, 2001). Most toxic exposures occur from indoor fungal growth related to excessive moisture. More research is required in this field to better understand the

relationship of fungal contamination, mycotoxin production on building substrates, and building related disease.

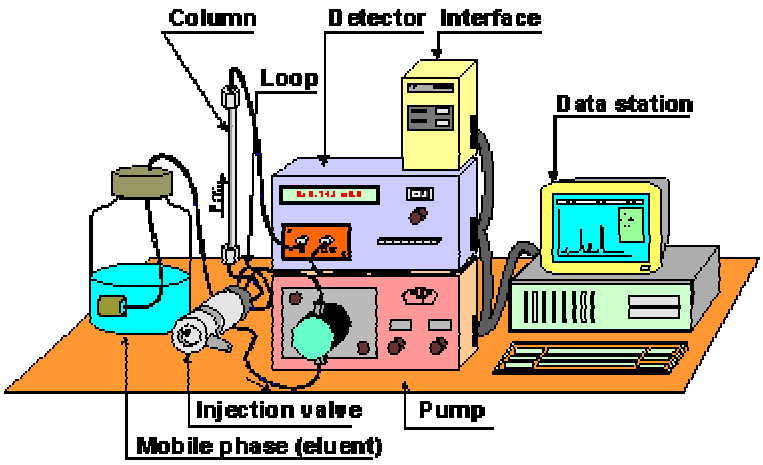
Because the substances (including mycotoxins) responsible for causing health symptoms in indoor environments are imperfectly known and cannot be detected directly, mycotoxin testing is subject to controversy. "Direct mycotoxin monitoring is [typically] only applicable where there is a clear environmental dominance of an individual toxin-producing species, or a special concern regarding a particular toxin or class of toxins" such as epidemiological studies (Summerbell, 1995).

Testing and Methods

Mycotoxin testing and analysis are still in a preliminary research phase. A general test for cytotoxicty has been suggested for a rough hazard assessment of indoor environments, though numerous limitations arise regarding this issue. One of the most significant problems associated with airborne mycotoxins testing is obtaining a suitable amount of spores from which the toxin can be extracted for chemical analysis (Microbial Diagnostics, Inc., 2002). The most sensitive tests available require hundreds of thousands of toxigenic spores, the presence of which basically precludes detection of toxins in the air in most situations (Miller, 2001). Aerotech Laboratories therefore suggest all mycotoxins sampling therefore be done through bulk, surface, or dust sampling (Aerotech, 2002). PCR amplification though has been successfully used in association with *Tricothecenes* to reduce the number of spores necessary for detection (Aerotech, 2002).

All mycotoxins tests are performed on representative samples as no analytical approaches can detect mycotoxins in real time (Microbial Diagnostics, Inc., 2002). Additionally, mold isolates may lose some strains for secondary metabolite production when held in the laboratory. Other limitations include the need to establish the total microflora (Nielsen, 2002).

High Performance Liquid Chromatography (HPLC)



HPLC has been the most important analytical method for mycotoxins since the late 1980s and start of the 1990s (Nielsen, 2002). High Performance Liquid Chromatography is a chemistry-based tool for quantifying and analyzing the amount of a chemical compound within a mixture of chemicals (Waters Corporation, 2002). The sample is dissolved in a solvent such that a liquid mobile phase is created. The mixture is forced through a chromatographic column under high pressure where separation occurs. This is conducted utilizing the fact that certain compounds have different migration rates given a particular column and mobile phase (HPLC: A User's Guide). A sensor, called a detector (UV, fluorescence, refractive index, conductivity, etc.), emits a response

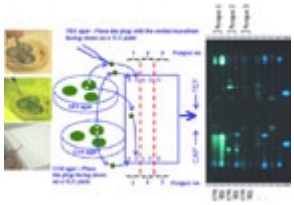
corresponding to the eluting sample compound to a data system (computer, integrator, or recorder), and subsequently signals a peak on the chromatogram. These peaks are correlated to specific mycotoxins. (For more detailed information, see Basic Liquid Chromatography, Textbook on High Performance Liquid Chromatography (HPLC) by Dr Yuri Kazakevich Dr. Harold McNair, 1996 (**http://hplc.chem.shu.edu/NEW/HPLC Book/**.)

New techniques for HPLC have improved separation, identification, purification, and quantification far beyond other techniques for mycotoxins analysis. Instrumentation has decreased in price and is getting easier to maintain. HPLCs are highly versatile, easily automated, and PCs can sample and do automated detection of target compounds. Significantly larger samples can be injected in a HPLC than a GC (Nielsen, 2002).

Thin Layer Chromatography (TLC)

TLC is the oldest of the chromatographic methods, requires less advanced and expensive equipment than other chromatographic methods, and a considerable sample speed can be achieved. After separation, target components can be detected visually or instrumentally under light or UV light, and can more or less be selectively colored for quantification. TLC is not sensitive enough for detecting TR (Nielsen, 2002).

Thin-layer chromatography (TLC) method (adopted from the Mycology Group, BioCentrum, Technical University of Denmark)

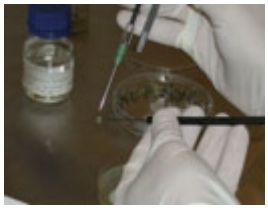


1. All work has to be done in a fumehood

2. Instruments.

1. TLC plates, Silicagel 60, Merck Art 5721 (not shown)
2. Griseofulvin in methanol
3. Chloroform/methanol solution (2:1).
4. Steel tube (i.d. 4 mm) for taking plugs and a Steel pipe for pushing out the plugs again.
5. Needle for transferring the plug
6. Standard for placing the plugs (optional)

2. From the center of a colony on CYA two agar plugs are cut using a stainless steel metal tube (i.d. 4 mm).



3. The agar plug mounted and wetted by a drop of chloroform/methanol (2:1).

3. The mycelium side is then gently pressed for a few seconds onto a TLC plate (Silicagel 60, Merck Art 5721) 2.5 cm from the bottom line.

4. One agar plug from YES is placed on the TLC plate with the agar side down towards the TLC plate placed 1 cm from where the CYA plugs were placed. When the liquid, extracted from the agar, from the plug is visible on the TLC plate the plug is removed.
5. The same procedure is done on the opposite side of the TLC plate (see Fig.)

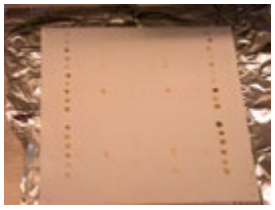
6. In the middle lane griseofulvin (10-100 µg/ml in methanol) is place on both sides as internal standard

7. The TLC plate is then eluted for 15 min. (or until it reaches half the plate height) in CAP (chloroform/acetone/2-propanol; 85/15/20) and the dried for 5 min. *Important that this is done before the TEF elution!*

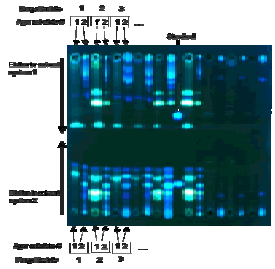
8. Plate after 1-2 minutes

Remember that there should be about 1 cm of solvent in the container, and that the paper should be wet. The solvent should be dragged up with the same speed.

Then the plate is eluted from the opposite side for 15 min in TEF (Toluene/ethyl acetate/90% formic acid; 5/4/1).



The TLC plate is then dried for 1 hour.



The plate under UV-light

Plate evaluation

- The plate is examined in daylight and under UV light (366 nm and 265 nm) and the spots are noted.
- Spray treatments: The plate is the sprayed with AlCl3 (20 % (w/v) AlCl3.6 H2O in 60 % ethanol) and heated at 130ºC for 8 min.

The secondary metabolites visible in daylight and under UV light are noted.

- The CAP side is then sprayed with $Ce(SO_4)_2$ (1 % in 3 M H_2SO_4)
  - The secondary metabolites visible in daylight and under UV light are noted.
- The TEF side is the sprayed with ANIS (0,5 % p-anisaldehyde in ethanol/conc. acetic acid/conc. sulphuric acid; 17/2/1) and heated at 130ºC for 8 min.
  - Secondary metabolites visible in daylight and UV light are noted.

Examples:

A TLC plate with *P. chrysogenum* and *P. viridicatum*

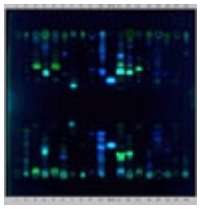


Plate seen at 254 nm after spray with AlCl3 (click to enlarge)

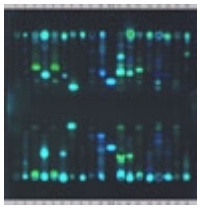


Plate seen at 366 nm after spray with AlCl3 (click to enlarge)

A TLC plate with P. citrinum, *P. crustosum* and *P. brevicompactum*

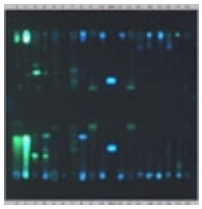


Plate seen at 366 nm (click to enlarge)

Plate at normal light after spray with $Ce(SO_4)_2$

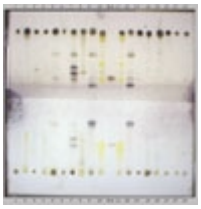
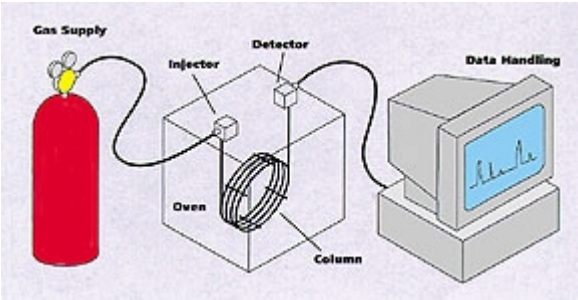


Plate at normal light after spray with anis aldehyde

A vast species library (such as the plate examples above) exists for identification.

Gas Chromatography (GC)



GC works similarly to other chromatographic methods. One or more high purity gases are supplied to the GC. One of the gases (called the carrier gas) flows into the injector, through the column, where the eluting compounds are separated, and then into the detector and data processing machine (Agilent Technologies).

Gas Chromatography used as the method of analysis for mycotoxin testing will always have to face instability problems during injection and chromatography, because mycotoxins are generally non volatile (Nielsen, 2002). To be suitable for GC analysis, a compound must have sufficient volatility and thermal stability. If all or some of a compound's molecules are in the gas or vapor phase at 400-450°C or below, and they do not decompose at these temperatures, the compound can probably be analyzed by GC (Start GC!). For TR, GC-MS (Mass Spectrometry) is the primary analytical method.

Capillary electrophoesis (CE)

CE separates molecules in the presence of an electric field inside a capillary. Molecules separate based on their charge-to-mass ratio (CE Resources Pte Ltd**.,** 2002).

CE separation power exceeds GC by several magnitudes and provides very fast separation speeds. Limitations with CE involving mycotoxins include that uncharged components cannot be separated unless a micellar CE is used, which greatly reduces

separation power. The small sample volumes, which must be loaded on the column, give detection limit problems even after concentration methods have been used (Nielsen, 2002).

ELISA (enzyme-linked immunosorbent assay)

ELISA is an "immunoassay technique involving the reaction of antigen and antibody in vitro…ELISA is a sensitive and specific assay for the detection and quantification of antigens or antibodies" (komabiotech.com/FAQ/immunology/ELISA.htm).

ELISA routinely used for detection of AFB_1, TB DON, ochratoxin A, and zearalenone in cereals, and is becoming an increasing important clean-up technique in combination with chromatographic detection methods (Nielsen, 2002).

Bioassays

Bioassays are some of the oldest and most primitive types of analysis for toxicity testing as they are conducted using biological organism agents as the 'sampling media'. The first assays for measuring Stachybotrys toxins, were rabbit skin tests, which later were shown as highly sensitive to TR. In vitro cultivated cell cultures of human or animal cell lines can provide the same sensitivity and specificity.

Cell cultures are the most common assay for cytotoxicity testing, and are very successful is detecting trichothecenes and indoor air organisms. The major problem with mammalian cell cultures is the need for highly sterile environments and frequent sub-cultivation, which is very time and material consuming. Using sperm cells, blood cells, or rings from chicken trachea where the movement of cilia can be monitored offer a more

simple solution to these problems. Brine shrimp assays have been extensively used for TR and aflatoxins (Nielsen, 2002).

Discussion

The extration of metaboloites is very difficult due to interfering materials and they may not become airborne at all if they are excreted into the material. The large amount of spores necessary along with the lack of validated sampling and clean-up procedures make mycotoxin sampling not feasible at this time. Charlie Wiles, executive director of the Indoor Air Quality Association, and an Aerotech Laboratories representative both advised against mycotoxins testing for indoor samples as not enough is known about mycotoxins and their effects (verbal communication, 2002). Mycotoxin testing may be warranted when an established health effect associated with known fungi and mycotoxin was established, and another health effect is discovered with an unknown causal/correlative agent. "Knowing the type of mold present or whether it produces toxins is not necessary to determine the appropriate method for clean up; …prudent health practice advises speedy clean-up and removal of heavily exposed populations from exposure as a first resort" (Davis, 2001).

APPENDIX:

Some Common Mycotoxins and the Organisms that Produce Them

Mycotoxin	Organism
Acetoxyscirpenediol	*Fusarium moniliforme, F. equiseti,* F. oxysporum , F. culmorum , F. avenaceum , F. roseum , and F. nivale
Acetyldeoxynivalenol	*Fusarium moniliforme, F. equiseti,* F. oxysporum , F. culmorum , F. avenaceum , F. roseum , and F. nivale
Acetylneosolaniol	*Fusarium moniliforme, F. equiseti,* F. oxysporum , F. culmorum , F. avenaceum , F. roseum , and F. nivale
Acetyl T-2 toxin	*Fusarium moniliforme, F. equiseti,* F. oxysporum , F. culmorum , F. avenaceum , F. roseum , and F. nivale
Aflatoxin	*Aspergillus flavus, A. parasiticus*
Aflatrem	*Aspergillus flavus*
Altenuic acid	*Alternaria alternata*
Alternariol	*Alternaria alternata*
Austdiol	*Aspergillus ustus*
Austamide	*Aspergillus ustus*
Austocystin	*Aspergillus ustus*
Avenacein [+1]	*Fusarium moniliforme, F. equiseti,* F. oxysporum , F. culmorum , F. avenaceum , F. roseum , and F. nivale
Beauvericin [+2]	*Fusarium moniliforme, F. equiseti,* F. oxysporum , F. culmorum , F. avenaceum , F. roseum , and F. nivale
Bentenolide	*Monographella nivalis*
Brevianamide	*Aspergillus ustus*
Butenolide	*Fusarium moniliforme, F. equiseti,* F. oxysporum , F. culmorum , F. avenaceum , F. roseum , and F. nivale
Calonectrin	*Fusarium moniliforme, F. equiseti,* F. oxysporum , F. culmorum , F. avenaceum , F. roseum , and F. nivale
C haetoglobosin	*Chaetomium globosum*
Citrinin	*Aspergillus carneus, A. terreus, Penicillium citrinum, P. hirsutum, P. verrucosum*
Citreoviridin	*Aspergillus terreus, Penicillium citreoviride*
Cochliodinol	*Chaetomium cochliodes*
Crotocin	*Acremonium crotocinigenum*
Cytochalasin E	*Aspergillus clavatus*

Cyclopiazonic acid	*Aspergillus versicolor*
Deacetylcalonectrin	*Fusarium moniliforme, F. equiseti,* F. oxysporum , F. culmorum , F. avenaceum , F. roseum , and F. nivale
Deoxynivalenol diacetate	*Fusarium moniliforme,* and F. nivale
Deoxynivalenol monoacetate	*Fusarium moniliforme,* F. culmorum , F. avenaceum , F. roseum , and F. nivale
Diacetoxyscirpenol	*Fusarium moniliforme, F. equiseti*
Destruxin B	*Aspergillus ochraceus*
Enniatins	*Fusarium moniliforme,* F. avenaceum , F. roseum , *F. solani,* and F. nivale
Fructigenin [+1]	*Fusarium moniliforme,* F. culmorum , F. avenaceum , *and* F. roseum
Fumagilin	*Aspergillus fumigatus*
Fumonisin B_1	*Fusarium moniliforme,* F. culmorum , F. avenaceum , and F. nivale
Fusaric acid	*Fusarium moniliforme*
Fusarin	*Fusarium moniliforme*
Gliotoxin	*Alternaria, Aspergillus fumigatus, Penicillium*
HT-2 toxin	*Fusarium moniliforme,* F. culmorum , F. avenaceum , and F. nivale
Ipomeanine	*Fusarium moniliforme,* F. culmorum , F. avenaceum , and F. nivale
Islanditoxin	*Penicillium islandicum*
Lateritin [+1]	*Fusarium moniliforme,* F. culmorum , F. avenaceum , and F. nivale
Lycomarasmin [+1]	*Fusarium moniliforme*
Malformin	*Aspergillus niger*
Maltoryzine	*Aspergillus spp.*
Moniliformin	*Fusarium moniliforme, F. equiseti,* F. oxysporum , F. culmorum , F. avenaceum , F. roseum , and F. nivale
Monoacetoxyscirpenol	*Fusarium moniliforme, F. equiseti,* F. oxysporum , F. culmorum , F. avenaceum , F. roseum , and F. nivale
Neosolaniol	*Fusarium moniliforme, F. solani,* F. culmorum , F. avenaceum , and F. roseum
Nivalenol	*Fusarium moniliforme, F. equiseti,* F. oxysporum , F. culmorum , F. avenaceum , F. roseum , and F. nivale
NT-1 toxin	*Fusarium moniliforme, F. equiseti,* F. oxysporum , F. culmorum , F. avenaceum . F. roseum . and F. nivale

NT-2 toxin	*Fusarium moniliforme, F. equiseti,* F. oxysporum , F. culmorum , F., F. solani, avenaceum , F. roseum , and F. nivale
Ochratoxin	*Aspergillus ochraceus, Penicillium viridictum*
Oxalic acid	*Aspergillus niger*
Patulin	*Aspergillus clavatus, Penicillium expansum, Botrytis, P. roquefortii, P. claviforme, P. griseofulvum*
Penicillic acid	*Aspergillus ochraceus*
Penitrem	*Penicillium crustosum*
Roridin E	*Myrothecium roridum, M. verrucaria, Dendrodochium spp., Cylindrocarpon spp., Stachybotrys spp.*
Rubratoxin	*Penicillium rubrum*
Rubroskyrin	*Penicillium spp.*
Rubrosulphin	*Penicillium viridicatum*
Rugulosin	*Penicillium brunneum, P. kloeckeri, P. rugulosum*
Sambucynin [+1]	*Fusarium moniliforme, F. equiseti,* F. oxysporum , F. culmorum , F. solani, F. avenaceum , F. roseum , and F. nivale
Satratoxins, F,G,H	*Stachybotrys chartarum, Trichoderma viridi*
Scirpentriol	*Fusarium moniliforme, F. equiseti,* F. oxysporum , F. culmorum , F. solani, F. avenaceum , F. roseum , and F. nivale
Slaframine	*Rhizoctonia leguminicola*
Sterigmatocystin	*Aspergillus flavus, A. nidulans, A. versicolor, Penicillium rugulosum*
T-1 toxin	*Fusarium moniliforme, F. equiseti,* F. culmorum , F. solani, F. avenaceum , F. roseum , and F. nivale
T-2 toxin	*Fusarium moniliforme, F. equiseti,* F. culmorum , F. solani, F. avenaceum , F. roseum , and F. nivale
Triacetoxyscirpendiol	*Fusarium moniliforme, F. equiseti,* F. avenaceum , F. roseum , and F. nivale
Trichodermin	*Trichoderma viride*
Trichothecin	*Trichothecium roseum*
T richoverrins	*Stachybotrys chartarum*
T richoverrols	*Stachybotrys chartarum*
Tryptoquivalene	*Aspergillus clavatus*
Verrucarin	*Myrothecium verrucaria, Dendrodochium spp., Stachybotrys chartarum*
Verruculogen	*Aspergillus fumigatus, Stachybotrys chartarum*
Viopurpurin	*Trichophyton spp., Penicillium viridicatum*
Viomellein	*Aspergillus spp., Penicillium aurantiogriseum, P. crustosum, P. viridicatum*

Viriditoxin	*Aspergillus fumigatus*
Xanthocillin	*Eurotium chevalieri*
Yavanicin [+1]	*Fusarium culmorum, F. graminearum, F. oxysporum, F. roseum,* F. moniliforme , F. avenaceum , F. equiseti , and F. nivale
Zearalenone	*Fusarium culmorum, F. graminearum, F. oxysporum, F. roseum,* F. moniliforme , F. avenaceum , F. equiseti , and F. nivale

Currently, surface, bulk, food and feeds, and air samples can be analyzed relatively inexpensively for the following mycotoxins

Aflatoxin

Aflatoxin is one of the most potent carcinogens known to man and has been linked to a wide variety of human health problems. The FDA has established maximum allowable levels of total aflatoxin in food commodities at 20 parts per billion. The maximum level for milk products is even lower at 0.5 parts per billion. Primarily Aspergillus species fungi produce aflatoxin.

Ochratoxin

Ochratoxin is primarily produced by species of Penicillium and Aspergillus. Ochratoxin is damaging to the kidneys and liver and is also a suspected carcinogen. There is also evidence that it impairs the immune system.

T-2 Toxin

T-2 Toxin is a tricothecene produced by species of Fusarium and is one of the more deadly toxins. If ingested in sufficient quantity, T-2 toxin can severely damage the entire digestive tract and cause rapid death due to internal hemorrhage. T-2 has been implicated in the human diseases alimentary toxic aleukia and pulmonary hemosiderosis. Damage caused by T-2 toxin is often permanent.

Fumonisin

Fumonisin is a toxin associated with species of Fusarium. Fumonisin is commonly found in corn and corn-based products, with recent outbreaks of veterinary mycotoxicosis occurring in Arizona, Indiana, Kentucky, North Carolina, South Carolina, Texas and Virginia. The animals most affected were horses and swine, resulting in dozens of deaths. Fumonisin toxin causes "crazy horse disease", or leukoencephalomalcia, a liquefaction of the brain. Symptoms include blindness, head butting and pressing, constant circling and ataxia, followed by death. Chronic low-level exposure in humans has been linked to esophageal cancer. The American Association of Veterinary Laboratory Diagnosticians (AAVLD) advisory levels for fumonisin in horse feed is 5 PPM.

Vomitoxin or Deoxynivalenol (DON)

Vomitoxin, chemically known as Deoxynivalenol, a tricothecene mycotoxin, is produced by several species of Fusarium. Vomitoxin has been associated with outbreaks of acute gastrointestinal illness in humans. The FDA advisory level for vomitoxin for human consumption is 1ppm.

Zearalenone

Zearalenone is also a mycotoxin produced by Fusarium molds. Zearalenone toxin is similar in chemical structure to the female sex hormone estrogen and targets the reproductive organs.

Other mycotoxins of clinical significance are as follows:

Citrinin

Citrinin is a nephrotoxin produced by Penicillium and Aspergillus species. Renal damage, vasodilatation, and bronchial constriction are some of the health effects associated with this toxin.

Alternariol

Alternariol cytotoxic compound derived from Alternaria alternata

Satratoxin H

Satratoxin H is a macrocyclic tricothecene produced by Stachybotrys chartarum, Trichoderma viridi and other fungi. High doses or chronic low doses are lethal. This toxin is abortogenic in animals and is believed to alter immune system function and makes affected individuals more susceptible to opportunistic infection.

Gliotoxin

Gliotoxin is an immunosuppressive toxin produced by species of Alternaria, Penicillium and Aspergillus.

Patulin

Patulin is a mycotoxin produced by Penicillium, Aspergillus and a number of other genera of fungi. It is believed to cause hemorrhaging in the brain and lungs and is usually associated with apple and grape spoilage.

Sterigmatocystin

Sterigmatocystin is a nephrotoxin and a hepatotoxin produced by Aspergillus versicolor. This toxin is also considered to be carcinogenic.

The following protocol / "tech tips" from Aerotech Laboratories is for bulk, surface, or dust vacuum mycotoxin sampling only. No method of standards for air sampling currently exists (though remember, 100,000 spores are necessary for analysis except for Trichothecenes).

Required Equipment for Sampling

Sterile Gloves

Ziploc Bags for Bulk Samples

Methanol Swabs for Surface Samples

DustChek™ Bags for Dust Samples

Standard Vacuum Cleaner (if dust sampling)

Sampling Methods for Mycotoxins in Bulk Samples

Bulk samples should be cut and aseptically removed from the source and placed in a clean, sterile container. The approximate weight should be 25-50 grams of sample.

When possible, select bulk samples with visible fungi contamination. Bulk materials that can be used for the determination of mycotoxins include such items as wallpaper, cardboard, wood, plasterboard, paper covered gypsum board, mineral wool, plaster, sand, linoleum, polyurethane insulation, pipe insulation and paint chips.

Sampling Methods for Mycotoxins in Surface Samples

1. Remove the swab from its wrapping and insert the swab into the vial of methanol. Ensuring that the cotton end is thoroughly saturated in the solvent.

2. Swab a calculated area and record on chain of custody. This will enable the laboratory to calculate concentrations based upon the area swabbed. If possible swab a 4" X 4" area.

3. Re-insert the swab into the methanol vial and snap the handle on the swab so the entire swab fits into the vial. Discard the broken handle of the swab. The cotton end of the swab should be totally immersed into the methanol in the vial.

4. Twist the cap tightly on the methanol vial and seal lid to vial with tape to prevent solvent spilling in shipment. Label sample vial appropriately and record on chain of custody.

Surface sampling is a nondestructive sampling technique, but may not be suitable for all types of surfaces.

Sampling Methods for Mycotoxins in Dust Samples

1. If using the dust collection cassette (DustChek™), clean vacuum hose attachment with warm soapy water, rinse with fresh water and dry thoroughly.

2. Choose an area of carpet or flooring to be sampled. The goal is to collect a significant amount of dust. Wet carpet should not be sampled by this method, as dust will not likely be drawn into the collection filter.

3. Attach collector nozzle to the hose attachment of the vacuum. (Remove the bottom end cap prior to attaching to hose.)

4. Remove top cap and set aside. Check to make sure filter is inserted into the nozzle head.

5. Vacuum for at least 2-5 minutes, to insure enough material is collected for analysis.

6. To prevent loss of material, tilt the nozzle upward before turning off vacuum. Place on the top cap to close off filter.

7. Remove nozzle from hose.

8. Gently push the filter out with your finger and place directly into a zip lock bag. Do not tap filter as the micro-fine dust is needed for analysis.

9. To take a second sample, wipe down the nozzle with an alcohol wipe, inside and out. Let dry for a minute and then insert a fresh filter.

When selecting an area to sample, choose an area where dust may have settled and accumulated. A sample of dust from a non-contaminated area should be collected for comparison studies and quality control purposes.

Quality Control

Multiple samples should be collected for comparison studies to include at a minimum: a complaint and non-complaint sample. There are no governmental or industrial regulations concerning allowable mycotoxins or toxigenic mold spores in indoor environments.

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