



Aspects of Toxin Production Implications for Health

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Mycotoxins (mold poisons)

- **Exotoxins** are secreted onto the substrate where molds grow
- **Relatively small molecules; products of secondary metabolism; not volatile, associated with particles**
- **More than 300 mold species have been found to produce one or more toxins**
- **Organisms affected vary (bacteria, other fungi, plants, animals)**
- **Spectrum of toxicity from highly potent (LD_{50} = less than a mg/kg) for verrucarin A, J, satratoxin, aflatoxins; to low (100 mg/kg) for others**



Location of Mycotoxins

- **Toxins are found in and on spores and mycelium of producers**
- **Toxins are found on fragments of spores and mycelia, and on small particles produced by molds**
- **Toxins are secreted unto the substrates where they grow and are found on dirt and dust particles that can be aerosolized**

Toxins associated with very small particles

- **Lab studies show large number of fungal and actinomycete fragments (30 nm to 1 μ diameter) are released together with spores from moldy surfaces at a ratio of about 500 to one (Górny et al., 2002, 2003; Cho et al. 2005)**
- **Field studies (New Orleans, S. Ohio) found fragment/spore ratios 1,000 for 0.3 μ , and 1,000,000 for 0.03 μ size fraction respectively Reponen et al. 2007**

Toxin Production by Molds Indoors

- **3 conditions for toxin production re:**
Stachybotrys chartarum, *Aspergillus versicolor*, *Chaetomium globosum*, *Aspergillus fumigatus*
 1. **a_w greater than 0.90**
 2. **Mixed cultures of microbes competing for niche**
 3. **Substrate that supports mold growth**

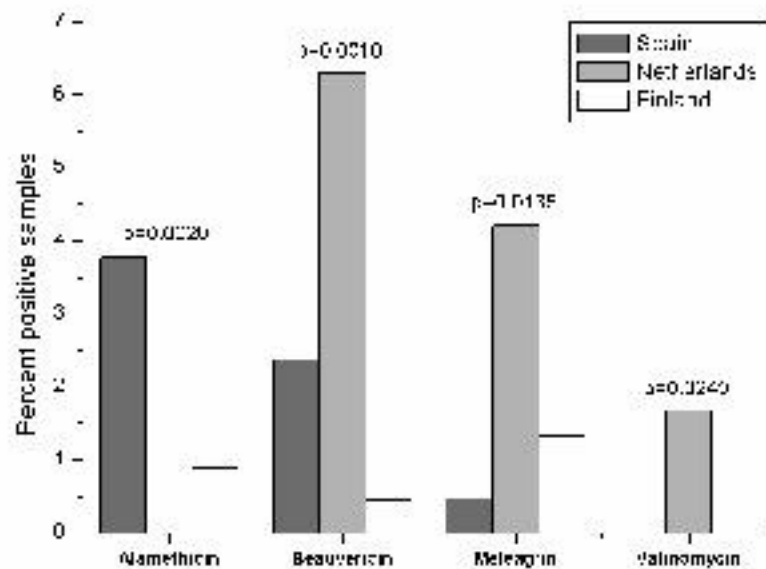
Nielsen 2003; Fox, Howlett 2008



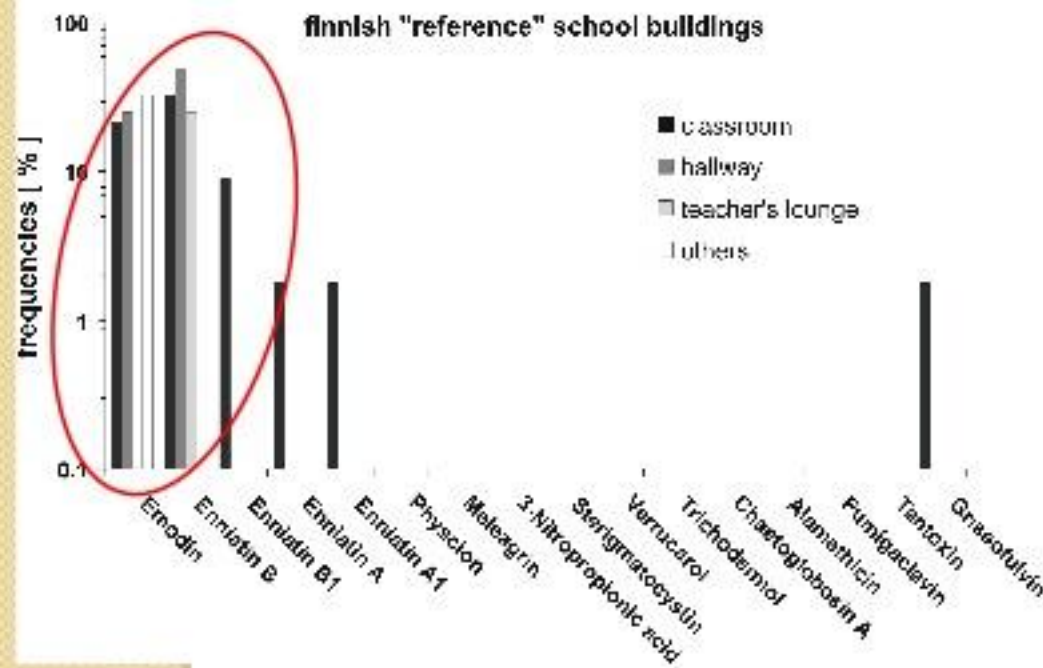
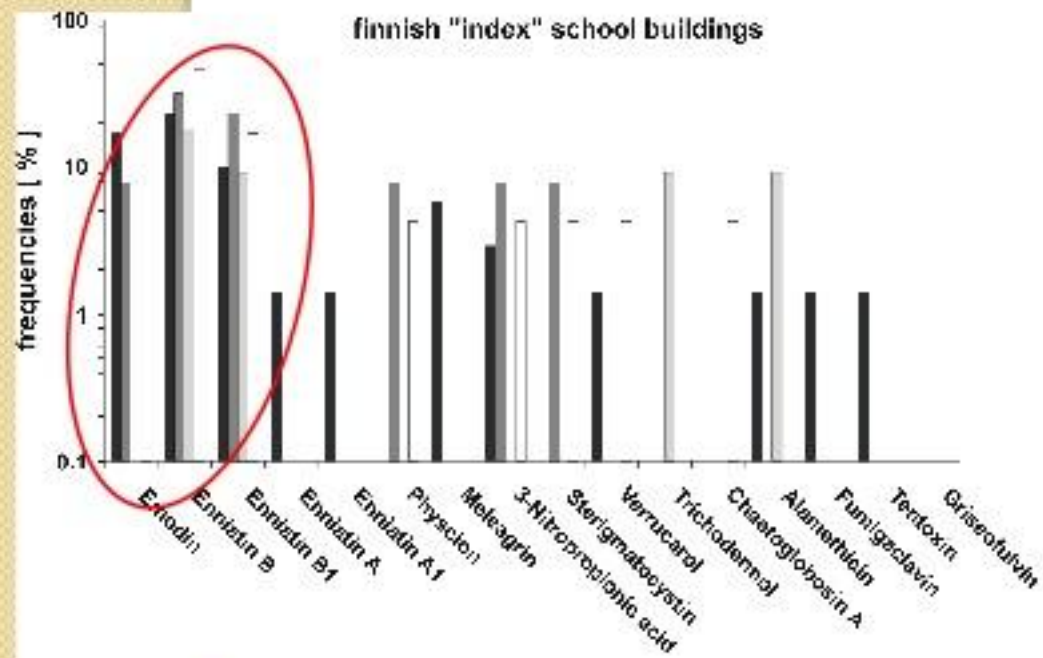
Research on microbial toxins in indoor environments (HITEA study)

- **Screening of occurrence of ~200 metabolites in indoor samples**
- **Sampling from mold damaged and control buildings, outdoor air**
- **Data combined with microbiological and health data**
- **At present, preliminary data available**

Occurrence of toxins in house dust of schools in Finland, Netherlands and Spain (HITEA)



Differences in frequencies of detection of toxins – that origin potentially from indoor sources - in settled dust (SDS) from school buildings in Spain, The Netherlands and Finland ($p<0.05$ according to Chi-Square Test are shown).



42-58% of samples positive

prevalence of metabolites <10% (other than emodin, enniatins)

some differences in metabolites profiles between countries

in general, greater variety of metabolites in index school buildings

certain indoor related toxins only present in index schools; not consistent between countries

'background' toxins related to outdoor sources?

Microbial metabolites in Finnish index and reference school buildings

'Background toxins' related to outdoor sources?

- o **emodin, physcion:**

produced by *Eurotium* sp., but also produced in some plants (vegetables, herbs)

- o **enniaticins, beauvericin:**

produced by *Fusarium* sp., commonly found in contaminated grain dusts (Hintikka et al. 2009)

→ plant material, mycotoxin containing dust, insects are plausible, 'moisture damage independent' sources for presence of metabolites in indoor environments

Occurrence of toxic bacterial and fungal metabolites on moldy building materials (Peitzsch et al. 2012)

Microbial metabolites (positive/total samples)	Detection of metabolites in 9 building material samples								
	4105	4106	4107	4108	4109	4110	4111	4112	4113
Fungal									
Chaetoglobosin A (8/9)	+	+	+	+		+	+	+	+
Emodin (5/9)	+	+		+		+			+
Meleagrins (9/9)	+	+	+	+	+	+	+	+	+
Roquefortine C (3/9)			+				+	+	
Stachybotrylactam (4/9)			+			+		+	+
Sterigmatocystin (6/9)	+	+		+	+			+	+
Trichodermol (1/9)								+	
Bacterial									
Monactin (3/9)					+	+		+	
Valinomycin (6/9)		+		+	+	+		+	+
Number of different metabolites per sample:	4	5	4	5	4	6	3	8	6

Summary of the toxin data



- ✓ a big variety of bioactive microbial metabolites and toxins was found in schools (n=18 in Finnish schools); geographical variation of metabolite patterns in schools over Europe
- ✓ prevalence of single compounds was rather low (*in particular for typical indoor related toxins*)
- ✓ few statistical significant associations of metabolite findings with damage status of the schools
 - *indoor production of microbial toxins likely to depend on severe dampness/moisture damage conditions and related microbial growth*
- ✓ outdoor sources seem to play a role in the presence of 'background' toxins (equally found in reference buildings)

Molds Differ in Ability to Make Toxins

- Not all genera or strains are toxigenic
- Species or strains within a genus differ in type and amount of toxins made
- Availability of nutrients, O₂, water, presence of competitors determine toxin production

Nielsen, 2002

- Stage of successional cycle in fungal community: 1°, 2°, 3° colonizers
- Toxin production gives ecological advantage

Fox , Howlett 2008



Habitat and Community Changes

- **Building moisture is often episodic, affects whether organisms live or die**
- **Food changes; dirt, dust accumulates; as organisms die they become mold food; building structures made from “was wood”, or paper products (i.e. gypsum board) get digested when damp or wet**
- **Conditions become favorable for new molds, other organisms: succession of species occurs, but their products, especially mycotoxins remain**

Growth Changes Substrate

- **Substrates are changed by microbial growth, can support new populations as new molecules become available, environmental conditions change**
- **Growth, sporulation, metabolic stage influence how microbes use substrate**
- **While populations change, the persistent molecules such as mycotoxins, persist on substrate or dust after organisms are no longer detectable**



Most significant toxin producers indoors

- **Produce very potent toxins or produce many toxins with varied effects**
- **Are frequently associated with growth of producer in buildings**
- **Producers of toxins are frequently found in wet or damp buildings**
- **There is significant chance of human exposure (new perspective regarding small particles)**



Important Toxigenic Molds in Damp Buildings

Nielsen KF, 2003

- ***Stachybotrys chartarum***
- ***Aspergillus versicolor***
- ***Chaetomium globosum*,
Chaetomium spp.**
- ***Aspergillus fumigatus***

Stachybotrys chartarum

- **Highly cytotoxic**
- **Biomass from areas with this mold contain higher quantities of 2° metabolites**
- **Strain S (30 – 40 % of isolates) produce highly toxic macrocyclic trichothecenes satratoxin H, G, F, iso-F, roridin L-2 (potent inhibitors of protein synthesis); roridin E epimers, hydroxy- epimer roridin E and L-2; 10-40 different spirocyclic drimanes; atranones and derivatives, simple trichothecenes, and stachylysin a protein, causes bleeding)**

Nielsen 2003

Stachybotrys chartarum

- **Strain A produces atranones (not cytotoxic to macrophages) that induce inflammation, and moderate inhibition of protein synthesis**

Pestka et al. 2008; Andersen et al., 2002; Nielsen 2003

- **Also produces spirocyclic drimanes, like Strain S; large quantities synthesized, broad spectrum of biological activity: immune disruption, enzyme inhibition, cytotoxicity, etc.** Nielsen, 2003



Implications of *S. chartarum* toxicity

- **Cytotoxicity: cell damage and death: at point of contact (readily absorbed from lung)**
- **Protein Synthesis Inhibition: affects repair of damaged tissues, healing; learning; enzyme production; antibody synthesis; growth**
- **Immune disruption: up-, down-regulation of immune system: In herd animals, exposure manifests first as increased susceptibility to infection**

Aspergillus versicolor

- Together with *Penicillium chrysogenum* most common species of indoor damp spaces
- Can grow on nutrient-poor substrate, i.e., concrete and plaster
- Consistently produces large amounts of carcinogenic sterigmatocystin on water-saturated materials (7 to 20 μ /cm²)

Nielsen 2003

Sterigmatocystin

- **Sterigmatocystin also found in 20% of household dust samples at levels up to 4 ng/g dust** Englehart et al., 2002
- **Sterigmatocystin (a precursor of aflatoxin B-1) is transformed to its carcinogenic form by cytochrome P₄₅₀ enzymes in liver and lung**
- **Is strong inhibitor of tracheal ciliary movement**
- **Highly inflammatory to lung cells**



Chaetomium globosum

- **Most common *Chaetomium* in damp buildings**
- **Produces highly cytotoxic mycotoxins chaetomins and chaetoglobosins**
- **These inhibit cell division and glucose transport**
- **Produces ten other uncharacterized metabolites**

Aspergillus fumigatus

- **This mold is infectious in immune-compromised individuals; currently most prevalent airborne fungal infection in developed world** Latgé 1999, 2001
- **Commonly isolated in moldy buildings, especially from dust**
- **“It has an amazing arsenal of biologically active metabolites including fumigaclavines (ergot alkaloids), fumitoxins, fumitremorgens, gliotoxins, tryptoquivalins and verruculgen.”** Nielsen 2003



Mycotoxins as Virulence Factors for Aspergillosis?

- **Disease occurs primarily in immunocompromised patients (HIV, chemotherapy, organ-transplant, cancer patients, etc.) but also in some occupants of moldy buildings.**
- **Healthy hosts' macrophages ingest and kills the spores of this mold from lung**
- **Many mycotoxins affect immune defenses, especially macrophage function and ciliary clearance, and mixed communities of molds tend to produce mycotoxins**

***Aspergillus fumigatus* and Gliotoxin**

- **Gliotoxin is one of most abundantly produced mycotoxins from this mold**
- **Suppresses immune function**
- **Inhibits macrophage and PMN function (host cellular defenses)**
- **Produces fatal invasive aspergillosis in immuno-competent mice**
- **Recovered from 93% of *A. fumigatus* cultures from cancer patients with presumptive invasive aspergillosis** Lewis et al., 2005

***A. Fumigatus* and ergot alkaloids**

Panaccione, Coyle, 2005

- **Four ergot alkaloids: fumigaclavine C, festuclavine, fumiclavine A, fumiclavine B (in order of abundance) associated with conidia**
- **Total ergot alkaloids can be > 1% of mass of conidia (under appropriate environmental conditions) on latex paint**
- **Ergot alkaloids negatively affect CV, nervous, reproductive, immune systems**
- **Effects from indoor exposure not studied**

Role of *A. fumigatus* toxins in other respiratory disease?

- **Gliotoxins**
- **Ergot alkaloids (fumigaclavins)**
- **Verruculogen**
- **Fumitremorgens**
- **Fumitoxins**

We know toxic endpoints from *in vitro* and *in vivo* role in damp building exposures other than facilitating infection not studied



Microbial Interactions and Toxin Production

- **On microbial level, competition can affect secondary metabolites such as toxins**
- **On human health level, increased production of toxins can have synergistic effects on inflammation, or increased immune disruption, or can affect virulence or bacterial survival (i.e., amoebae sequestration of bacteria)**

Respiratory Health

- **Most studied: direct access, route of entry**
- **Inflammation common denominator for allergy, toxicity**
- **Allergic asthma implicated for mold**
- **Non-allergic asthma also implicated for mold**
- **Symptoms similar; inflammatory mediators similar; IgE allergic antibodies do not have role in non-allergic asthma**

Meta-analyses of epidemiological studies in IOM

Fisk, Lei-Gomez, Mendel, 2007

- **Conclusion: building dampness and mold associated with 30 to 50% (CI 95% 1.34-1.75) increases in variety of respiratory and asthma-related outcomes**
- **“Consistent and relatively strong associations of dampness and health effects strongly suggest causation by dampness-related exposures”** Fisk et al., 2007
“Research has not yet determined causal agents” IOM, 2004

Attributable Respiratory Illness: Dampness, Mold

Fisk, Lei-Gomez, Mendel, 2007

- **Upper respiratory tract symptoms 38% increase (OR 1.54 [1.33-1.78] for all exposed**
- **Cough 53% increase (OR 1.79 [1.57-2.03] for all exposed**
- **Wheeze 80% increase (OR 1.65 [1.48-1.83] for all exposed**
- **Fraction of current asthma attributable to dampness and mold : 21% (CI =12-29%)**

New Onset Asthma From Dampness and Mold

- **IOM (2004): insufficient number of studies for a conclusion of causality**
- **Studies of new asthma when subjects exposed to mold in homes and workplaces leads to conclusion that exposure causes asthma (Pekkanen et al., 2007; Jaakkola, Hwang, Jaakkola 2005; Jaakkola et al., 2002; Cox-Ganser et al. 2005; Iossifova et al., 2009; Park et al., 2008; Cox-Ganser et al. 2009; Karvala et al., 2010. etc.)**



Other Illness Associated with Mold/Mold Products

Limited number of epidemiological studies indicate evidence for:

- **Sarcoidosis**
- **Various Rheumatoid Problems**
- **Nervous System Effects**
- **Immune Dysfunction**
- **Eczema**
- **Lung Hemorrhage in Infants**

Limitations of Epidemiological Studies

- **No effect is found unless it is specifically studied (absence of evidence is not evidence of absence)**
- **Association shown by single studies; Inference of causality is judgment call: requires a body of work:**
 - **Conclusions of sufficient association depends on consistency in a number of well designed studies**
- **Measures of *exposure* are the biggest problem**



Possible Role in Non-allergic Asthma: of Mice and Men

- Human genome, mouse genome for some genes, gene interactions responsible for asthma, identified, similar
- Expression of genes in mouse lung in response to mycotoxins and glucans from molds from built environment yields inflammatory cytokines and mucin
- Signs of inflammation asthma-like Miller et al 2010
- Indication for Causal Toxin Role

Guttation Droplets



Guttation Droplets

- Active excretion of water and dissolved materials from plants and fungal mycelia:
- Several *Aspergillus* and *Penicillium* species excrete water-soluble mycotoxins in guttation droplets on aerial mycelia
- High levels of ochratoxin from *P. nordicum* and *P. verrucosum* Gareis, Gareis 2007 and *A. niger* and *A. ochraceous* Muñoz et al., 2011 depending on culture medium and age of culture

Agents of Exposure?

- Can fine aerosol droplets from guttation be a means of exposure to mycotoxins?
- Highly polarized mycotoxins are soluble in water

Ochratoxin A Gareis 2007

Satratoxins Harach et al.1982; Black et al. 2006

Fumonisin, Nevalinol, DON

Lauren,

Ringrose 1997