

# TOXICOLOGY AND RISK ASSESSMENT OF MYCOTOXINS

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## I. BACKGROUND

The presence of fungi or mold in buildings that are damaged by water is an area attracting public health attention, since it has been shown that some genera of molds are capable of producing a chemically diverse group of potentially toxic metabolites known as “mycotoxins”. Molds are ubiquitously found both indoors and outdoors and grow on a plethora of surfaces;<sup>1</sup> however, molds that are capable of producing mycotoxins require specific growth conditions to do so, Table 1.<sup>2</sup> The most commonly implicated genus of mold for producing mycotoxins in water-damaged buildings is *Stachybotrys*.

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1. S. Gravesen et al., *Microfungal Contamination of Damp Buildings-Examples of Risk Constructions and Risk Materials*, 107 ENVIRON. HEALTH PERSPECT. 505, 505-08 (1999).

2. C. Grant et al., *The Moisture Requirements of Moulds Isolated from Domestic Dwellings*, 25 INTL. BIODETERIOR. 259, 259-84 (1989).

A myriad of health problems, ranging from nonspecific indoor air quality complaints in adults to specific cases of pulmonary hemorrhage in infants, have been attributed to mycotoxins.<sup>3</sup> Advocates for mold-induced illnesses in humans point to the identified toxicological data obtained from mycotoxins (mold metabolites) in animal models; however, these studies are based on ingestion or inoculation of large doses of the toxic agents into the test animals, Tables 2, 3, and 4. Moreover, no studies have shown that inhalation of mold spores, and possibly mycotoxins, at levels expected in mold-contaminated indoor environments are responsible for causing measurable health effects.<sup>4</sup> Historically, cases of mycotoxin-induced illnesses (mycotoxicoses) have resulted from mass poisonings of livestock or humans that ingested large quantities of contaminated foodstuffs.

The consumption of foodstuff contaminated with mycotoxins can have deadly outcomes. However, this condition drastically differs from the claims that residing in a building contaminated with mold can cause measurable health problems. The former case results in the internalization of the toxin versus the latter case where there is only the potential for inhalation of minute amounts of mold, and possibly, mycotoxins. At present, the weight-of-evidence in the medical literature indicates that mold exposures occurring in contaminated buildings do not present an overt health hazard. It is advisable, however, that mold be removed from indoor environments, along with other possible irritants, such as dust mites, bacteria, animal dander, pollen, etc.

## II. MOLD EXPOSURE AND RISK ASSESSMENT

The production of mycotoxins (e.g. aflatoxin, ergotamine, ochratoxin, patulin, rubratoxin, trichothecenes) is highly dependent on the type of mold and the environmental conditions.<sup>5</sup> In strains implicated in mycotoxicosis, not all produce detectable mycotoxins.<sup>6</sup> Therefore, the presence of molds is not proof of the presence of toxins. A case in point is that of *Stachybotrys chartarum* which is a cellulose-decaying fungus with worldwide distribution. It grows

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3. F. Fung et al., *Stachybotrys, a Mycotoxin-Producing Fungus of Increasing Toxicologic Importance*, 36 CLIN. TOXICOL. 79, 79-86 (1998).

4. C. A. Robbins et al., *Health Effects of Mycotoxins in Indoor Air: A Critical Review*, 15 APPL. OCCUP. ENVIRON. HYG. 773, 773-84 (2000); A. I. Terr, *Stachybotrys: Relevance to Human Disease*, 87 ANN. ALLERGY ASTHMA IMMUNOL. 57, 57-63 (2001).

5. C. Grant et al., *The Moisture Requirements of Moulds Isolated from Domestic Dwellings*, 25 INTL. BIODETERIOR. 259, 259-84 (1989); K. M. Hendry & E. C. Cole, *A Review of Mycotoxins in Indoor Air*, 38 J. TOXICOL. ENVIRON. HEALTH 183, 183-98 (1993).

6. J. A. Chapman, *Stachybotrys Chartarum (Chartarum = Atra = Alternans) and Other Problems Caused by Allergenic Fungi*, 24 ALLERGY ASTHMA PROC. 1, 1-7 (2003).

well at room temperature and with humidity above 93% and can produce different types of macrocyclic trichothecenes, potent inhibitors of protein and DNA synthesis.<sup>7</sup> As noted by Persad,<sup>8</sup> route of exposure plays a key role in the development of disease. Direct administration of a large quantity of *Stachybotrys chartarum* spores into the lungs of rats has been shown to cause pulmonary inflammation and hemorrhage.<sup>9</sup> However, when exposed to surfaces heavily tainted with this mold, and conditions of high airflow, mice did not experience any adverse pulmonary effects.<sup>10</sup> These reports demonstrate the potential for an adverse outcome after receiving a high dose of mold spores versus the lack of effect from even heavy exposure to mold spores, respectively. The latter case is clearly more relevant for establishing risk assessments based on the presence of molds in buildings, since exposure to molds is not likely to result in a dose. Furthermore, mycotoxins are not volatile and when they are identified in samples, it is usually from those obtained from inert dust or building materials.<sup>11</sup> Therefore, the actual exposure may be greatly exaggerated, especially for molds, such as *Stachybotrys*, whose spores are produced in a slimy mass under conditions of high humidity.<sup>12</sup> If inhalation was to occur, it is most probable that mycotoxins would be inhaled with airborne particulates, such as dust or dried out fungal components that have been agitated.<sup>13</sup> However, since mycotoxins are confined to spores, it is doubtful that they frequently reach the lower airways due to

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7. Y. Rosenstein & C. Lafarge-Frayssinet, *Inhibitory Effect of Fusarium T2-Toxin on Lymphoid DNA and Protein Synthesis*, 70 TOXICOL. APPL. PHARMACOL. 283, 283-88 (1983).

8. A. S. Persad et al., *Mold in the Literature: Evaluating Human Health Risks from Mold-Contaminated Buildings*, 2 COLUMNS-MOLD 6, 6-7, 54-55 (2003).

9. C. Y. Rao et al., *Reduction of Pulmonary Toxicity of Stachybotrys Chartarum Spores by Methanol Extraction of Mycotoxins*, 66 APPL. ENVIRON. MICROBIOL. 2817, 2817-21 (2000).

10. C. K. Wilkins et al., *Respiratory Effects in Mice Exposed to Airborne Emissions from Stachybotrys Chartarum and Implications for Risk Assessment*, 83 PHARMACOL. TOXICOL. 112, 112-19 (1998).

11. M. A. Andersson et al., *Bacteria, Molds, and Toxins in Water-Damaged Building Materials*, 63 APPL. ENVIRON. MICROBIOL. 387, 387-93 (1997); W. A. Croft et al., *Airborne Outbreak of Trichothecene Toxicosis*, 20 ATMOS. ENVIRON. 549, 549-52 (1986); K. C. Ehrlich & L. S. Lee, *Mycotoxins in Grain Dust: Method for Analysis of Aflatoxins, Ochratoxin A, Zearalenone, Vomitoxin, and Secalonic Acid D*, 67 J. ASSOC. OFF. ANAL. CHEM. 963, 963-67 (1984); J. D. Miller et al., *Fungi and Fungal Products in Some Canadian Houses*, 24 INT. BIODETERIOR. 103, 103-20 (1988); M. Nikulin et al., *Stachybotrys Atra Growth and Toxin Production in Some Building Materials and Fodder under Different Relative Humidities*, 60 APPL. ENVIRON. MICROBIOL. 3421, 3421-24 (1994).

12. E.-L. Hintikka & M. Nikulin, *Airborne Mycotoxins in Agricultural and Indoor Environments*, Supp. 4 INDOOR AIR 66, 66-70 (1998).

13. M. S. Palmgren & L. S. Lee, *Separation of Mycotoxin-Containing Sources in Grain Dust and Determination of Their Mycotoxin Potential*, 66 ENVIRON. HEALTH PERSPECT. 105, 105-08 (1986); D. T. Wicklow & O. L. Shotwell, *Intrafungal Distribution of Aflatoxins among Conidia and Sclerotia of Aspergillus Flavus and Aspergillus Parasiticus*, 29 CAN. J. MICROBIOL. 1, 1-5 (1983).

size limitations, considering the depth of particle penetration is inversely proportional to size. The upper airways trap particles of 10 – 60  $\mu\text{m}$ , while particles of 2 – 4  $\mu\text{m}$  in diameter can reach the alveoli. As detailed in Table 5, mold spores generally have dimensions that prevent them from being respired into the smaller airways and alveoli.<sup>14</sup>

### III. MOLD DETECTION & LEVELS IN AMBIENT AIR

Indoor environments are replete with various microorganisms including bacteria and molds, along with their potentially irritating products, including endotoxins and mycotoxins, respectively.<sup>15</sup> Generally, the presence of bacteria exceeds that of fungal species;<sup>16</sup> however, the majority of building-related health claims implicate only molds as the causative agents. This might be explained, at least partly, by the fact that molds can form visible colonies while other organisms may remain undetectable to the unaided eye. The extent to which molds are responsible for compromising the health of inhabitants is debatable, considering the quantity of substances present, the multitude of health complaints set forth, and the lack of association for buildings that contain mold versus control buildings. In nearly all cases, the complaints voiced are of a symptomatic nature, devoid of any clear, underlying medical explanation.<sup>17</sup> Of these, many have been collectively categorized into syndromes, e.g. sick building syndrome (SBS), indicating that the cause is unknown.

SBS is a commonly applied diagnosis, which is often abused and misinterpreted to denote headaches, dizziness, fatigue, and eye irritation associated with a building.<sup>18</sup> It has been shown that subjective factors, like mental stress, play a strong role in the

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14. D. H. LARONE, *MEDICALLY IMPORTANT FUNGI: A GUIDE TO IDENTIFICATION* (3rd ed., ASM Press 1995).

15. M. A. Andersson et al., *Bacteria, Molds, and Toxins in Water-Damaged Building Materials*, 63 *APPL. ENVIRON. MICROBIOL.* 387, 387-93 (1997); E.-L. Hintikka & M. Nikulin, *Airborne Mycotoxins in Agricultural and Indoor Environments*, Supp. 4 *INDOOR AIR* 66, 66-70 (1998); J. E. Cone & D. Shusterman, *Health Effects of Indoor Odorants*, 95 *ENVIRON. HEALTH PERSPECT.* 53, 53-59 (1991); R. E. Dales & D. Miller, *Residential Fungal Contamination and Health: Microbial Cohabitants as Covariates*, 107 *ENVIRON. HEALTH PERSPECT.* 481, 481-83 (1999).

16. E.L. Hintikka & M. Nikulin, *Airborne Mycotoxins in Agricultural and Indoor Environments*, Supp. 4 *INDOOR AIR* 66, 66-70 (1998); Health and Welfare Canada Working Group on Fungi and Indoor Air, *Significance of Fungi in Indoor Air: Report of a Working Group*, 78 *CAN. J. PUBLIC HEALTH* S1, S1-S14 (1987).

17. P. Wargoeki et al., *Subjective Perceptions, Symptom Intensity and Performance: A Comparison of Two Independent Studies, Both Changing Similarly the Pollution Load in an Office*, 12 *INDOOR AIR* 74, 74-80 (2002).

18. Y. J. Tsai & M. E. Gershwin, *The Sick Building Syndrome: What Is It When It Is?*, 28 *COMPR. THER.* 140, 140-44 (2002).

perceived suffering of subjects. In one study, 2,160 subjects in 67 offices were evaluated for psychological stress and building-related symptoms. It was concluded that employees experiencing more physical and mental stress reported a higher prevalence of these symptoms compared to controls.<sup>19</sup>

Additional factors warrant further investigation when identifying causative agents and SBS. For example, the levels of humidity in a building can not only promote mold, bacteria, and dust mite growth, but also affect the rate of off-gassing of formaldehyde from indoor building materials, formation of acids and salts from sulfur and nitrogen dioxide, and the formation of ozone.<sup>20</sup> Many of the upper airway complaints attributed to mold exposure may in fact be due to dust mites, which are notorious allergens, or bacteria, as these are all potential sources of confounding when examining mold and moisture and adverse health effects.<sup>21</sup>

There are no established levels of exposure for which molds can compromise health in humans, as the daily outdoor air spore counts vary considerably both seasonally and geographically in the U.S., Table 6.<sup>22</sup> Most studies typically present a comparison between outdoor and indoor mold counts. Generally, these values are reported as colony forming units per cubic meter of air (CFU/m<sup>3</sup>). This method entails the collection of air samples (e.g. Andersen sampler), which are then grown on agar media for several days. After the incubation period, the plates are inspected, and the colonies of mold are identified by macroscopic and/or microscopic analysis and expressed as CFU/m<sup>3</sup> for each respective genus of mold.<sup>23</sup>

The collection of air samples for the same specimen can result in variations up to 1,000-fold based on the sampler type.<sup>24</sup> Thus, it is very important to utilize a unified protocol when assessing mold levels in ambient air, especially when comparing control and mold-

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19. P. L. Ooi & K. T. Goh, *Sick Building Syndrome: An Emerging Stress-Related Disorder?*, 26 INT. J. EPIDEMIOL. 1243, 1243-49 (1997).

20. A. V. Arundel et al., *Indirect Health Effects of Relative Humidity in Indoor Environments*, 65 ENVIRON. HEALTH PERSPECT. 351, 351-61 (1986).

21. M. A. Andersson et al., *Bacteria, Molds, and Toxins in Water-Damaged Building Materials*, 63 APPL. ENVIRON. MICROBIOL. 387, 387-93 (1997); R. E. Dales & D. Miller, *Residential Fungal Contamination and Health: Microbial Cohabitants as Covariates*, 107 ENVIRON. HEALTH PERSPECT. 481, 481-83 (1999); D. Menzies et al., *Aeroallergens and Work-Related Respiratory Symptoms among Office Workers*, 101 J. ALLERGY CLIN. IMMUNOL. 38, 38-44 (1998).

22. National Allergy Bureau, *NAB: Pollen & Mold Counts*, (2002) at <http://www.aaaai.org/nab/pollen.stm>.

23. A. A. Andersen, *New Sampler for the Collection, Sizing, and Enumeration of Viable Airborne Particles*, 76 J. BACTERIOL. 471, 471-84 (1958).

24. C. A. Hunter et al., *Mould in Buildings: The Air Spora of Domestic Dwellings*, 24 INT. BIODETERIOR. 81, 81-101 (1988).

contaminated buildings. Single samples are typically obtained for the former versus multiple samples for the latter,<sup>25</sup> a situation that will almost assuredly result in an overestimation of the mold counts in contaminated buildings. Other factors that need to be considered when interpreting data of mold spore samples, include the conditions the sampling was performed under, e.g. normal room conditions versus more aggressive measures, such as vacuuming, carpeting type, pets, dust control measures, and humidification.<sup>26</sup> Finally, the dimensions of spores vary considerably and thus may be an important factor when attempting to quantify some species, considering larger spores will settle more quickly than smaller ones.<sup>27</sup>

The largest study performed to date with a unified protocol was completed by Shelton.<sup>28</sup> This study analyzed 9,619 indoor mold samples and 2,407 outdoor mold samples collected across the U.S. over a three-year period. This study found that the most common culturable airborne fungi, both indoors and outdoors and in all seasons and regions of the U.S., were *Cladosporium*, *Penicillium*, and *Aspergillus*.<sup>29</sup> No statistically significant association was observed between any common fungal type and reported health complaints. The most commonly identified genera of mold and the average mold counts from indoor samples are shown in Table 7.

Many studies focus on correlating individual symptoms of an illness from residing or working in buildings with various genera of molds that can potentially produce trichothecenes, including: *Fusarium*, *Stachybotrys*, and *Trichoderma*.<sup>30</sup> Shelton determined that when *Stachybotrys* was present indoors, the average concentration was 12 CFU/m<sup>3</sup> [95% confidence interval (CI), 12 – 118 CFU/m<sup>3</sup>]; however, this genus was only detected in 6% of the

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25. C. Grant et al., *The Moisture Requirements of Moulds Isolated from Domestic Dwellings*, 25 INTL. BIODETERIOR. 259, 259-84 (1989).

26. S. R. Hirsch & J. A. Sosman, *A One-Year Survey of Mold Growth inside Twelve Homes*, 36 ANN. ALLERGY 30, 30-38 (1976); P. P. Kozak, Jr. et al., *Factors of Importance in Determining the Prevalence of Indoor Molds*, 43 ANN. ALLERGY 88, 88-94 (1979); W. R. Solomon, *A Volumetric Study of Winter Fungus Prevalence in the Air of Midwestern Homes*, 57 J. ALLERGY CLIN. IMMUNOL. 46, 46-55 (1976).

27. S. Gravesen et al., *Microfungal Contamination of Damp Buildings-Examples of Risk Constructions and Risk Materials*, 107 ENVIRON. HEALTH PERSPECT. 505, 505-08 (1999); A. Hyvärinen et al., *Comparison of the Indoor Air Quality in Mould Damaged and Reference Buildings in a Subarctic Climate*, 9 CENT. EUR. J. PUBLIC HEALTH 133, 133-39 (2001).

28. B. G. Shelton et al., *Profiles of Airborne Fungi in Buildings and Outdoor Environments in the United States*, 68 APPL. ENVIRON. MICROBIOL. 1743, 1743-53 (2002).

29. *Id.*

30. M. Mahmoudi & M. E. Gershwin, *Sick Building Syndrome. III. Stachybotrys Chartarum*, 37 J. ASTHMA 191, 191-98 (2000); C. M. Scheel et al., *Possible Sources of Sick Building Syndrome in a Tennessee Middle School*, 56 ARCH. ENVIRON. HEALTH 413, 413-17 (2001).

buildings studied.<sup>31</sup> Furthermore, human exposure to *Stachybotrys* species has not resulted in any significant association of health problems in buildings with culturable levels of *Stachybotrys* species and those without.<sup>32</sup>

#### IV. CONCLUSIONS

At present, the weight-of-evidence in the medical literature indicates that mold exposures occurring in residential and commercial buildings are not likely to result in significant health hazards. It is advisable, however, that mold be removed from indoor environments, along with other possible irritants, such as dust mites, bacteria, animal dander, pollen, etc. The production of mycotoxins is highly dependent on the type of mold and the indoor environmental conditions. Therefore, the presence of molds alone is not proof of the presence of toxins. Indoor environments are replete with various microorganisms including bacteria and molds, along with their potentially irritating products. Generally, the presence of bacteria exceeds that of fungal species; however, the majority of building-related health claims allege only mold as the causative agent. This might be explained, at least partly, by the fact that molds can form visible colonies while other organisms may remain undetectable to the unaided eye. The extent to which molds are responsible for compromising the health of inhabitants is debatable, considering the quantity of other substances present, the diversity of health complaints set forth, and the lack of epidemiological data to validate an association between mold exposure and significant adverse health effects.

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31. B. G. Shelton et al., *Profiles of Airborne Fungi in Buildings and Outdoor Environments in the United States*, 68 APPL. ENVIRON. MICROBIOL. 1743, 1743-53 (2002).

32. *Id.*











