

MYCOTOXINS: MECHANISMS OF TOXICITY AND METHODS OF DETECTION FOR IDENTIFYING EXPOSED INDIVIDUALS

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I. INTRODUCTION

The concern over exposure to mold and human disease dates to antiquity. In recent centuries, the majority of mass poisonings attributed to molds have been due to the ingestion of fouled foods that contain secondary metabolites of molds also known as mycotoxins. Nearly 400 mycotoxins have been discovered to date and are generally categorized into groups based on structural similarities.¹ Some of the most common types of mycotoxins that can cause health problems in animals and humans are the aflatoxins, fumonisins, trichothecenes, ochratoxins, and zearalenones.

Molds are ubiquitously found both indoors and outdoors. Currently, there are several regulatory guidelines established for the allowable levels of specific mycotoxins in foods; however, there are no guidelines established for the safe-level of exposure to molds that are present indoors. Part of the ambiguity in determining a safe-level of mold exposure stems from the seasonal and geographic fluctuations that occur in levels of mold spores and the lack of correlation between sampling values and reported health effects.² Moreover, a considerable amount of controversy surrounds the relevance of measurable levels of mold from indoor environments and adverse health effects based on exposures that occur from inhalation.³

Some genera of fungi are capable of eliciting adverse health outcomes independent of the effect of mycotoxins. Such ailments include aspergilloma by *Aspergillus* species (spp.) and subcutaneous nodules by *Cladosporium* spp. However, these conditions are generally seen in immunocompromised patients, such as individuals infected with the human immunodeficiency virus. Examples of diseases that may result from mold infection are listed in Table 1.

The literature is rife with studies on the adverse health effects of mycotoxins in animals; however, these studies are generally limited to inoculation of the test animal with the toxin or intratracheal instillation of large numbers of spores from genera of mold known to produce mycotoxins. For example, in one study, mice were administered spores from a mycotoxin-producing strain of *Stachybotrys atra* in the amount of 1×10^5 spores via the intratracheal route.⁴ All animals exhibited inflammatory changes

1. J. W. Bennett & M. Klich, *Mycotoxins*, 16 CLIN. MICROBIOL. REV. 497, 498 (2003).

2. R. E. Gots et al., *Indoor Health: Background Levels of Fungi*, 64 AIHA J. (FAIRFAX, VA) 427, 429 (2003).

3. *Id.* at 435.

4. M. Nikulin et al., *Effects of Intranasal Exposure to Spores of Stachybotrys Atra in Mice*, 35 FUNDAM. APPL. TOXICOL. 182, 184 (1997).

in the lungs. However, by adjusting this value for a 70-kg adult, Revankar noted the lack of relevance of this exposure (i.e. an inhaled dose of 3.5×10^9 spores) versus ambient levels of mold found indoors.⁵ More recently, Sava's findings suggest that neurotoxicity in mice occurs after injecting animals with a hepatotoxic dose of the mycotoxin, rubratoxin B.⁶ Although the administered dose (5 mg/kg) has repeatedly been shown to cause severe hemorrhagic necrosis of the liver, the authors failed to present indices of liver viability/toxicity (i.e. serum transaminases), which are paramount in the interpretation of such studies, considering that neurological sequelae are common following compromised liver function.

Mycotoxins have received extensive attention as the possible causative agents in buildings where molds are present because of their established potential to cause adverse health effects when ingested with contaminated foodstuffs. Although the relationship between indoor mold exposure and adverse health outcomes is unclear, thousands of insurance claims have been filed across the country and mold remediation has become a thriving business as a result. Interestingly, the peer-reviewed scientific literature is replete with studies addressing indoor mold and mycotoxins as causative agents in human disease as exemplified with *Stachybotrys* in Table 2; however, in addition to the shortcomings listed in Table 2, sources of confounding (i.e. dust mites, animal dander, off-gassing of volatile compounds, etc.) are routinely not excluded and diagnostic tests to confirm mycotoxins in human tissues are generally lacking.

Because of the level of uncertainty between measurable levels of molds and adverse health outcomes, biotechnology companies have been actively involved in developing sensitive and specific methodologies to detect mycotoxins in human tissues. The present article will provide a brief overview for several clinically relevant mycotoxins and their mechanisms of toxicity, followed by a discussion of the most commonly utilized methodologies for detecting mycotoxins in test samples.

5. S. G. Revankar, *Clinical Implications of Mycotoxins and Stachybotrys*, 325 AM. J. MED. SCI. 262, 271 (2003).

6. V. Sava et al., *Distribution of Oxidative DNA Damage and Oxyguanosine Glycosylase Activities across Brain Regions of Mice Injected with a Single Dose of Rubratoxin-B*, THE SOCIETY FOR NEUROSCIENCE 33RD ANNUAL MEETING, Program No. 669.4 (2003), <http://sfn.scholarone.com/itin2003/>.

II. MECHANISMS OF TOXICITY FOR SELECT MYCOTOXINS

An understanding of the mechanism(s) of toxicity provides a more definitive basis for biological plausibility between exposure and outcome, a cornerstone in the establishment of causation. The following mycotoxins have been extensively studied and the pathways that give rise to organ-specific pathologies have been described for many animal species, including humans. However, the damage produced in animals from the administration of high-doses of a particular mycotoxin does not correlate with mycotoxin-induced pathologies in humans, given the small amounts of mycotoxins that are typically present in air or food. Moreover, specific mycotoxins, such as aflatoxins that were suspected of increasing the risk of liver cancer have since been shown to pose no increased risk when consumed at the estimated daily levels.⁷ Finally, it is important to keep in mind that the dose determines the poison when reading the following established pathways for mycotoxin-induced tissue damage.

A. Aflatoxins

Aflatoxins were first identified as the probable toxin that destroyed more than 100,000 turkey poultts (Turkey X disease) in England in the early 1960s.⁸ These compounds consist of over a dozen members with aflatoxin B₁ being the most extensively studied, due to its propensity to bind to DNA upon activation by the cytochrome P450 enzyme family (Figure 1).⁹ The four major aflatoxins (aflatoxin B₁, B₂, G₁, and G₂) are named based on their fluorescence under blue or green light and their relative mobility during thin-layer chromatography (TLC).¹⁰ The hydroxylated metabolites of aflatoxins B₁ and B₂ (aflatoxins M₁ and M₂) may be found in milk or milk products from livestock that have ingested contaminated feed.¹¹ DNA adducts with aflatoxin B₁-8,9-epoxide are generally removed by the nucleotide excision repair pathway; however, if unrepaired, they may cause GC to TA transversions (i.e. a point mutation in which a purine is substituted by a pyrimidine or vice versa) and subsequent cellular changes that may lead to

7. WHO, *Evaluation of Certain Mycotoxins in Food*, WHO TECHNICAL REPORT SERIES 906 1, 16 (2002).

8. D. M. Kuhn & M. A. Ghannoum, *Indoor Mold, Toxicogenic Fungi, and Stachybotrys Chartarum: Infectious Disease Perspective*, 16 CLIN. MICROBIOL. REV. 144, 150-51 (2003).

9. S. G. Revankar, *Clinical Implications of Mycotoxins and Stachybotrys*, 325 AM. J. MED. SCI. 262, 264 (2003).

10. J. W. Bennett & M. Klich, *Mycotoxins*, 16 CLIN. MICROBIOL. REV. 497, 500 (2003).

11. WHO, *Evaluation of Certain Mycotoxins in Food*, WHO TECHNICAL REPORT SERIES 906 1, 9 (2002).

cellular transformation.¹² Recent exposures are most reliably detected by measuring the levels of the B₁-N7 adduct in urine.¹³ Several analytical methods are available for the detection of aflatoxins, including enzyme-linked immunosorbent assays (ELISAs), TLC, and liquid chromatography, or a combination of immuno-affinity columns and TLC or liquid chromatography.¹⁴ It is estimated that human consumption of aflatoxins ranges from 0 to 30,000 ng/kg/day with an average of 10 to 200 ng/kg/day.¹⁵

B. Fumonisin

Fumonisin were first discovered in 1988.¹⁶ The most extensively studied member of this class is fumonisin B₁. Fumonisin are rather unique in the world of mycotoxins in that they are water-soluble, which may account for their late discovery in mycotoxin research. In all animal species studied, fumonisin are poorly absorbed from the digestive tract and are rapidly distributed and eliminated.¹⁷ Numerous species-specific pathologies have been attributed to fumonisin-contaminated feed, including: leukoencephalomalacia (hole in the head syndrome) in equine and pulmonary edema and hydrothorax in swine.^{18, 19, 20} These compounds have been shown to have carcinogenic potential in animal models and are the only known inhibitors of ceramide kinase, a key enzyme involved in inflammatory cascades (Figure 2). Fumonisin are generally detected by purification with immuno-affinity columns followed by TLC or liquid chromatography; however, rapid screening tests based on TLC and ELISAs have been developed.²¹ It has been estimated that consumption of fumonisin B₁ by humans in the U.S. is approximately 80 ng/kg/day.²²

12. J. W. Bennett & M. Klich, *Mycotoxins*, 16 CLIN. MICROBIOL. REV. 497, 500 (2003).

13. *Id.* at 501

14. WHO, *Evaluation of Certain Mycotoxins in Food*, WHO TECHNICAL REPORT SERIES 906 1, 11 (2002).

15. S. G. Revankar, *Clinical Implications of Mycotoxins and Stachybotrys*, 325 AM. J. MED. SCI. 262, 264 (2003).

16. J. W. Bennett & M. Klich, *Mycotoxins*, 16 CLIN. MICROBIOL. REV. 497, 503 (2003).

17. WHO, *Evaluation of Certain Mycotoxins in Food*, WHO TECHNICAL REPORT SERIES 906 1, 19 (2002).

18. J. W. Bennett & M. Klich, *Mycotoxins*, 16 CLIN. MICROBIOL. REV. 497, 503 (2003).

19. W. F. Marasas et al., *Leukoencephalomalacia in a Horse Induced by Fumonisin B₁ Isolated from Fusarium Moniliforme*, 55 ONDERSTEEPOORT. J. VET. RES. 197, 197-203 (1988).

20. L. R. Harrison et al., *Pulmonary Edema and Hydrothorax in Swine Produced by Fumonisin B₁, a Toxic Metabolite of Fusarium Moniliforme*, 2 J. VET. DIAGN. INVEST. 217, 217-21 (1990).

21. WHO, *Evaluation of Certain Mycotoxins in Food*, WHO TECHNICAL REPORT SERIES 906 1, 23 (2002).

22. *Id.* at 25

C. Trichothecenes

Trichothecenes are one of the most recognized groups of mycotoxins, since they are the primary by-products of the mold species *Stachybotrys atra*. Trichothecenes constitute a family of over sixty compounds. Probably the most well-known member of this family is T-2 toxin because of its historic role in mass poisonings among livestock and humans from the consumption of contaminated food products and its potential use as a biological warfare agent. T-2 toxin is readily metabolized by the gut microflora of mammals to several metabolites. HT-2 toxin is a primary metabolite in the gut and is absorbed into the blood after ingestion of T-2 toxin. Metabolism continues in the liver (with biliary excretion), resulting in a substantial combined first-pass effect in the gut and liver.²³ Trichothecenes are direct acting compounds, unlike aflatoxin B₁ that requires metabolic activation. The mechanism of toxicity is generally by disruption of protein synthesis, more specifically by direct inhibition of peptidyltransferase in the large ribosomal subunit (Figure 3).²⁴ The primary effects of perturbed protein synthesis from T-2 toxin are seen in the immune system, and include changes in leukocyte counts, delayed hypersensitivity, depletion of selective blood cell progenitors, and depressed antibody formation.²⁵ TLC and liquid chromatography are the methods of choice for trichothecene detection; however, ELISAs have been developed for many members of this family.²⁶ The total intake of T-2 toxin and HT-2 toxin from dietary sources has been estimated at 7.6 and 8.7 ng/kg/day, respectively.²⁷

D. Ochratoxins

Ochratoxin A was discovered in 1965 and was later isolated from corn stuffs in the U.S.²⁸ This compound has been shown to induce acute tubular necrosis in all animal species studied.²⁹ Concern over human exposure stems from the extended half-life of this compound, which is greatest in humans than in other species. For example, the serum half-life of ochratoxin A varies among species, as follows: 24 — 39 hours in mice, 55 — 120 hours in rats, 72 — 120 hours in pigs,

23. *Id.* at 43

24. J. W. Bennett & M. Klich, *Mycotoxins*, 16 CLIN. MICROBIOL. REV. 497, 505 (2003).

25. WHO, *Evaluation of Certain Mycotoxins in Food*, WHO TECHNICAL REPORT SERIES 906 1, 44 (2002).

26. *Id.* at 47

27. *Id.* at 48

28. J. W. Bennett & M. Klich, *Mycotoxins*, 16 CLIN. MICROBIOL. REV. 497, 504 (2003).

29. *Id.*

510 hours in one macaque monkey, and 840 hours (35 days) in a human volunteer.³⁰ Ochratoxin A has been suggested as a causative agent of Balkan Endemic Nephropathy; however, more descriptive studies are needed to exclude other causative agents.³¹ Ochratoxin A disrupts several cellular functions, including ATP production; however, its toxicity is generally attributed to its inhibitory effects on the enzyme involved in the synthesis of the aminoacyl-tRNAs containing phenylalanine (Figure 4).³² Ochratoxin A can be detected in a wide range of products, including coffee beans.³³ Moreover, it has been readily detected in human blood and serum samples from individuals in Canada, Sweden, West Germany, and Yugoslavia.³⁴ Both TLC and liquid chromatography with fluorescent detection are used to identify ochratoxin A; however, ELISAs are also available.³⁵ The European Commission's Scientific Committee on Food has recommended that levels of ochratoxin A be reduced to below 5 ng/kg/day.³⁶ However, the estimated daily consumption of ochratoxin A from foodstuffs is approximately 6 ng/kg.³⁷

E. Zearalenones

Zearalenone is categorized as a mycotoxin; however, its toxicity is much lower than the previously mentioned compounds. For instance, the 50% lethal dose in female rats is greater than 10 g/kg.³⁸ A more appropriate categorization for this compound would be as a nonsteroidal estrogen or mycoestrogen because of its estrogenic-like properties (Figure 5), which have been reported in swine with as little as 1 mg/kg.³⁹ The levels of zearalenone in foodstuffs are currently not controlled by regulatory agencies; however, it has been estimated that the safe-level of exposure in humans is below 50 ng/kg/day.⁴⁰

30. WHO, *Evaluation of Certain Mycotoxins in Food*, WHO TECHNICAL REPORT SERIES 906 1, 29 (2002).

31. *Id.* at 30

32. J. W. Bennett & M. Klich, *Mycotoxins*, 16 CLIN. MICROBIOL. REV. 497, 504 (2003).

33. *Id.*

34. *Id.*

35. WHO, *Evaluation of Certain Mycotoxins in Food*, WHO TECHNICAL REPORT SERIES 906 1, 31 (2002).

36. J. W. Bennett & M. Klich, *Mycotoxins*, 16 CLIN. MICROBIOL. REV. 497, 507 (2003).

37. WHO, *Evaluation of Certain Mycotoxins in Food*, WHO TECHNICAL REPORT SERIES 906 1, 32 (2002).

38. J. W. Bennett & M. Klich, *Mycotoxins*, 16 CLIN. MICROBIOL. REV. 497, 507 (2003).

39. *Id.*

40. *Id.*

III. TECHNIQUES FOR MYCOTOXIN DETECTION

Several chemical and biological detection systems exist for the determination of mycotoxins (Table 3). T-2 toxin is one of the most extensively studied of the trichothecenes and several biological systems have been developed for detecting this toxin and other members of the trichothecene family (Table 4). Although these methods are useful for the determination of mycotoxin quantity, they lack specificity and mycotoxin identification is generally performed with one of the following physicochemical or immunological methods.⁴¹

A. Thin-Layer Chromatography

TLC is the most commonly utilized physicochemical test because more than one mycotoxin can be detected for each test sample. TLC is based on the separation of compounds by how far they migrate on a specific matrix with a specific solvent. The distance that a compound will travel is a unique identifier for specific compounds, and a retention factor (R_f) has been determined for most mycotoxins. As with any detection system, a positive control containing purified mycotoxins must be ran in parallel to ensure accuracy, since different chemicals can have a similar R_f (Figure 6).

B. Immunological Assays

Small molecules, such as mycotoxins, are not immunogenic and are known as haptens or molecules that will not stimulate antibody production by themselves. However, antibodies can be produced for a specific mycotoxin by conjugating it to a protein carrier, which causes the mycotoxin to become immunogenic.⁴² Animals produce several different types of antibodies that will recognize various regions of foreign particles, including antigens (a substance capable of stimulating an immune response) and haptens, when present on a carrier macromolecule. The various forms of antibodies include polyclonal and monoclonal types. Polyclonal antibodies react with multiple antigens or haptens on a foreign compound, whereas monoclonal antibodies react only with specific antigens or haptens.⁴³ Currently, both polyclonal and monoclonal antibodies have been developed that are available for identifying several types of

41. R. M. Eppley, *Methods for the Detection of Trichothecenes*, 58 J. ASSOC. OFF. ANAL. CHEM. 906, 907 (1975).

42. J. M. Fremy & E. Usleber, *Policy on Characterization of Antibodies Used in Immunochemical Methods of Analysis for Mycotoxins and Phycotoxins*, 86 J. AOAC INT. 868, 868-71 (2003).

43. *Id.*

mycotoxins in test samples by utilizing the ELISA (Figure 7) and immuno-affinity chromatography (IAC) (Figure 8).

IV. CONCLUSIONS

Despite the advances in methodologies for detecting mycotoxins, the problem of determining their causative role in health problems from indoor exposures will still persist, considering that presence of mycotoxins in the blood, serum, urine, etc., does not necessarily reflect inhalation exposure and more often than not represent mycotoxins consumed in foodstuffs. Carefully designed studies and proper assessment of exposure is needed to determine the effects of mycotoxins on human health.

