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FEATURE REVIEW ARTICLE

Risk from Inhaled Mycotoxins in Indoor Office and Residential Environments

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Mycotoxins are known to produce veterinary and human diseases when consumed with contaminated foods. Mycotoxins have also been proposed to cause adverse human health effects after inhalation exposure to mold in indoor residential, school, and office environments. Epidemiologic evidence has been inadequate to establish a causal relationship between indoor mold and nonallergic, toxigenic health effects. In this article, the authors model a maximum possible dose of mycotoxins that could be inhaled in 24 h of continuous exposure to a high concentration of mold spores containing the maximum reported concentration of aflatoxins B_1 and B_2, satratoxins G and H, fumitremorgens B and C, verruculogen, and trichoverrols A and B. These calculated doses are compared to effects data for the same mycotoxins. None of the maximum doses modeled were sufficiently high to cause any adverse effect. The model illustrates the inefficiency of delivery of mycotoxins via inhalation of mold spores, and suggests that the lack of association between mold exposure and mycotoxicoses in indoor environments is due to a requirement for extremely high airborne spore levels and extended periods of exposure to elicit a response. This model is further evidence that human mycotoxicoses are implausible following inhalation exposure to mycotoxins in mold-contaminated home, school, or office environments.

Keywords Aflatoxin, Fumitremorgen, Satratoxin, Tremorgen, Trichoverrol, Verruculogen

Mycotoxins have long been of interest to toxicologists because of their presence in animal feed and human food, and because of their potential for use as therapeutic drugs and biological warfare agents. Due to the widespread potential for significant exposure to mycotoxins in foods, the main focus of research outside of warfare agents has been related to ingestion of these compounds. Inhalation studies and other traditional toxicologic methods have been employed for biological warfare research purposes, and inhalation of mycotoxins has also been investigated to a limited extent in occupational and agricultural settings by clinicians, epidemiologists, and occupational health researchers.

Recently, mycotoxins have been suggested as agents of toxicity and adverse human health effects as a consequence of inhalation exposure in nonagricultural indoor environments. In support of that hypothesis, information from widely varying areas of research have been combined with anecdotal reports to produce stories in the media and litigation involving claims of injury due to 'toxic mold' in indoor environments. However, both rigorous epidemiologic study and application of dose-response principals have been lacking (Centers for Disease Control and Prevention [CDC] 2000; Hardin, Kelman, and Saxon 2003; Kuhn and Ghannoum 2003; Page and Trout 2001; Robbins et al. 2000; Terr 2001).

Although dose-response data concerning inhalation of mycotoxins are limited, information is sufficient to model upper limit doses in a theoretical, high-exposure environment. Burge (1996) estimated the time needed to absorb 1 ng of a mycotoxin (satratoxin H) at several exposure levels. However, that model included only one mycotoxin and did not include consideration of a dose at which an adverse effect might reasonably be expected. Here, we report an expansion of Burge's approach to include additional mycotoxins and comparisons to effect levels following in vivo animal exposure. Because mycotoxins are not volatile and do not evaporate from the mold spore or substrate particle (Pasanen et al. 1993; Schiefer 1990; Tuomi et al. 2000), inhalation exposure to mycotoxins requires inhalation of mycotoxin-containing mold or dust particles. Therefore, like Burge, we based exposure estimates on inhalation of mold spores containing mycotoxin.

The musty odor associated with mold is caused by volatile compounds generated during active mold growth. These compounds, which are different from the mycotoxins, may be annoying but are not toxic at concentrations produced by mold growth

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in office and residential environments and therefore were not modeled (Korpi et al. 1999). The model also does not address the antigenic properties of molds.

METHODS

Calculation of Potential Human Doses

The dose of mycotoxin inhaled by an individual in a 1-day (24-h) exposure was calculated as follows:

$$D = \frac{C_{\text{mycotoxin}} \times N \times \text{BR} \times \text{FR} \times \text{BA}}{\text{WT}} \quad [1]$$

where D is the dose of mycotoxin (μg/kg day), $C_{\text{mycotoxin}}$ is concentration of mycotoxin per spore (μg/spore), N is the number of spores/m^3 of air, BR is the breathing rate (m^3/day), FR is the fraction of inhaled spores retained in the lungs, BA is the fraction of mycotoxin that is released and biologically available, and WT is the body weight (kg) of the individual exposed. A maximal airborne mold spore concentration (N) of 200,000 spores/m^3 was assumed based on our experience collecting air samples in indoor environments with abundant visible surface mold. This airborne concentration is consistent with extreme values reported in indoor environments (Hunter et al. 1988; Shelton et al. 2002). Dose estimates are presented based on standard US Environmental Protection Agency (EPA) default values for adult males (breathing rate [BR] of 15.2 m^3/day and body weight [WT] of 78.1 kg) (EPA 1997) because they yielded higher ng/kg dose estimates than corresponding default values for adult females (breathing rate [BR] of 11.3 m^3/day and body weight [WT] of 65.4 kg). The fraction of spores retained (FR) and mycotoxin bioavailability (BA) both were assumed to be 100% as upper limits. With these assumptions, the maximum dose of mycotoxin inhaled by a hypothetical individual continuously exposed for 24 h is:

$$D_{\max}\ (\mu\text{g/kg day}) = \frac{C_{\text{mycotoxin}}(\mu\text{g/spore}) \times 200{,}000\ \text{spores/m}^3 \times 15.2\ \text{m}^3\text{/day} \times 1 \times 1}{78.1\ \text{kg}} \quad [2]$$

Mycotoxin Content of Spores

To establish $C_{\text{mycotoxin}}$ for equation [2], all relevant Dialog databases[1] were searched for data from peer-reviewed articles that could be used to calculate the amount of mycotoxin per spore. Mycotoxins included in the model were those for which concentration per spore data were available and that are produced under at least some conditions by molds known to grow indoors on building materials (these molds do not necessarily produce the modeled mycotoxins when growing indoors), and for which effect levels were also available. The mycotoxins that met these requirements for modeling were aflatoxins (AFs) B_1 and B_2, satratoxins G and H, fumitremorgens (fumitremorgens B and C and verruculogen), and trichoverrols A and B. When more than one published source reported data from which concentrations per spore could be calculated, we modeled the highest reported mycotoxin concentration.

Effect Levels

Inhalation exposure data were used when available to identify effect levels for comparison. However, human and animal data with inhalation exposure are limited and when they were unavailable, comparison doses were derived by extrapolating from other routes of administration.

CALCULATIONS

Published Maximal Mycotoxin Content of Spores (Table 1)

Aflatoxins B_1 and B_2

Seven environmental isolates of *Aspergillus flavus* (five recovered from indoor air samples, one from insulation material, and one from an air filter) and a known aflatoxin-positive laboratory *A. flavus* strain (NRRL-3251, National Center for Agricultural Utilization Research, Peoria, IL) were grown on building materials (ceiling tiles, wallpaper, and HVAC filters) and in broth culture using seven defined media (Ren, Ahearn, and Crow 1999). By high-performance liquid chromatography (HPLC) analysis, NRRL-3251 and five of the environmental isolates produced detectable aflatoxin in at least one of the media, whereas two of the isolates failed to produce aflatoxin in all conditions. Neither the environmental isolates nor NRRL-3251 produced aflatoxins when grown on building materials.

Ren, Ahern, and Crow (1999) quantified AFB_1 and AFB_2 concentrations in the conidia of one environmental isolate (931014) and the NRRL-3251 strain. AFB_2 was not detected in conidia from 931014 grown in any of the media; concentrations of AFB_1 ranged from $(0.03 \pm 0.02) \times 10^{-8}$ μg/spore in malt extract to $(3.3 \pm 0.6) \times 10^{-8}$ μg/spore in Czapek yeast autolysate (CY). With NRRL-3251, concentrations of AFB_1 ranged from $(0.15 \pm 0.08) \times 10^{-8}$ μg/spore in sucrose yeast extract to $(48 \pm 3.6) \times 10^{-8}$ μg/spore in glucose yeast extract; concentrations of AFB_2 ranged from none detected (with two media) to $(3.6 \pm 1.4) \times 10^{-8}$ μg/spore with glucose yeast extract. The highest published per spore concentrations of AFB_1 and AFB_2 are those for NRRL-3251 in glucose yeast extract broth, and these values were used for modeling.

Satratoxins G and H

Spores from two strains of *Stachybotrys atra* (now designated *S. chartarum*) grown on rice flour agar were extracted and analyzed for mycotoxin content (analytical methods not defined) (Nikulin et al. 1997). Strain 29 (s. 29) was described as "nontoxic" and did not produce detectable amounts of satratoxin G (SG) or satratoxin H (SH). Strain 72 (s. 72, Agricultural Research

[1] Biosis Previews, CA Search–Chemical Abstracts, Derwent Biotechnology Resource, Elsevier Biobase, EMBASE, Enviroline, General Science Abstracts, JICST–EPlus–Japanese Science and Technology, MEDLINE, New England Journal of Medicine, NIOSTIC, PASCAL, SCIENCE, SciScearch 1974–1989, SciSearch 1990–present, Toxfile.

TABLE 1
Maximum published concentrations of mycotoxins

Mycotoxin	Maximum μg/spore	Fungal source	Reference
Aflatoxin B1	4.8×10^{-7}	*A. flavus*	Ren, Ahearn, and Crow 1999
Aflatoxin B2	3.6×10^{-8}	*A. flavus*	Ren, Ahearn, and Crow 1999
Satratoxins G + H	14×10^{-8}	*S. chartarum*	Nikulin et al. 1997
Fumitremorgen B (HPLC analysis)	9.0×10^{-10}	*A. fumigatus*	Land et al. 1994
Fumitremorgen C (HPLC analysis)	1.2×10^{-8}	*A. fumigatus*	Land et al. 1994
Verruculogen (HPLC analysis)	2.2×10^{-9}	*A. fumigatus*	Land et al. 1994
Fumitremorgen B + verruculogen (TLC analysis)	8.0×10^{-8}	*A. fumigatus*	Land et al. 1994
Trichoverrols A + B	1.5×10^{-9}	*S. chartarum*	Sorenson et al. 1987

Centre, Jokioinen, Finland) was described as 'highly toxic' and spores contained 4×10^{-8} μg SG and 10×10^{-8} μg SH/spore. These are the highest published per spore concentrations of SH and SG, and because the effects level chosen for comparison is derived from dosing with these s. 72 spores, the combined total of 14×10^{-8} μg/spore was used for modeling.

Fumitremorgens

Ten strains of *Aspergillus fumigatus* isolated from Swedish sawmills and previously shown to produce tremorgenic mycotoxins were cultured on yeast extract sucrose medium (Land et al. 1994). Conidia from seven strains were analyzed by thin-layer chromatography (TLC) for gliotoxin and for fumitremorgens (fumitremorgen B and verruculogen combined); conidia from four strains were analyzed by HPLC for TR-2 and gliotoxin and for fumitremorgen B, fumitremorgen C, and verruculogen. No gliotoxin was detected by TLC or by HPLC. By TLC analysis, fumitremorgen concentrations in the seven isolates ranged from 0.6×10^{-8} to 8.0×10^{-8} μg/spore (mean 2.3×10^{-8} μg/spore). By HPLC analysis, fumitremorgen B concentrations in the four isolates ranged from 0.5×10^{-9} to 0.9×10^{-9} μg/spore (mean 0.7×10^{-9} μg/spore); fumitremorgen C and verruculogen were not detected in conidia from three isolates, whereas the fourth isolate (strain B, the only isolate represented in both TLC and HPLC analyses) contained 11.6×10^{-9} μg fumitremorgen C/spore and 2.2×10^{-9} μg verruculogen/spore (and 0.6×10^{-9} μg fumitremorgen B/spore). Concentrations used for modeling were, fumitremorgen B + verruculogen, 8.0×10^{-8} μg/spore (TLC analysis of strain A); fumitremorgen B, 0.9×10^{-9} μg/spore (HPLC analysis of strain K); fumitremorgen C, 11.6×10^{-9} μg/spore (HPLC analysis of strain B); and verruculogen, 2.2×10^{-9} μg/spore (HPLC analysis of strain B).

Trichoverrols A and B

Laboratory cultures of *S. atra* (*S. chartarum*) (Budapest 1 and Debrecen 1132 from the Veterinary Medical Research Institute, Hungarian Academy of Sciences, Budapest) were grown on sterile rice, autoclaved, and dried. Aerosolized samples were collected on glass-fiber filters (Sorenson et al. 1987). Microscopic analysis of the collected dust indicated its composition was 85% spores and 6% hyphal fragments, with the balance unidentified. Dust from seven replicate aerosol samples was extracted and analyzed by HPLC for trichoverrols A and B (T_T). None was detected in three samples, whereas the concentration in the four remaining samples ranged from 3.1×10^{-3} to 4.5×10^{-3} (mean 3.5×10^{-3}) μg T_T/mg dust. Assuming that 100% of the T_T was contained in the spores, and applying Burge's assumption that the mass of *S. atra* spores is 2.8×10^{-7} mg/spore (Burge 1996), the T_T concentration per spore (based on the sample with the highest measured T_T concentration) can be estimated as:

$$\mu g\ T_T/\text{spore} = \frac{2.8 \times 10^{-7}\ \text{mg/spore} \times 4.5 \times 10^{-3}\ \mu\text{g}\ T_T/\text{mg dust}}{0.85} = 1.48 \times 10^{-9}\ \mu\text{g}\ T_T/\text{spore}$$

Effects Reported from Laboratory Data (Table 2) and Regulatory-Derived Levels

Aflatoxin B_1

One approach to developing a comparison dose is to calculate the potential dietary intake at permitted levels of aflatoxin contamination in foods. Aflatoxin cannot be totally excluded from the food supply because *Aspergillus* species and other aflatoxin-producing fungi cannot be totally excluded from food products, principally corn and nuts, while in the field and in storage. The US Food and Drug Administration (FDA) established an action level of 20 ppb (20 ng/g) of total aflatoxins (B_1, B_2, G_1, G_2) in all food products (except milk, for which there is a separate action level of 0.5 ppb aflatoxin M_1) (CAST 2003); for peanuts, the US Department of Agriculture (USDA) Agricultural Marketing Service defines 15 ppb and below as "negative for aflatoxin"

TABLE 2
Mycotoxins: Effects levels in animals

	Effect level	Notes
Aflatoxin B_1	5.6 μg/kg	The inhalation dose of AFB_1 that produced no statistically significant reduction in alveolar macrophage phagocytic activity in rats (Jakab et al. 1994)
Satratoxins G + H	6.4×10^{-3} μg/kg	Pulmonary inflammation in mice after six intranasal doses of s. 72 *S. chartarum* spores, each spore dose delivering this dose of SG and SH (Nikulin et al. 1997)
Fumitremorgens	60 μg/kg	Intravenous dose of fumitremorgen A that produced "perceptible tremor in a few mice" but no mortality (Yamazaki, Suzuki, and Kukita 1979)
Verruculogen	60 μg/kg	Intravenous dose of fumitremorgen A that produced "perceptible tremor in a few mice" but no mortality (Yamazaki, Suzuki, and Kukita 1979)
Trichoverrols	2.0 μg/kg	Estimated dose of T-2 toxin retained by rats following 10-min inhalation exposure at the NOEL concentration of 1.0 mg T-2/m^3 (Creasia et al. 1990)

(7 CFR 996.11, as cited in USDA 2003). According to US food consumption data from the USDA for 1997 (the most recent year reported) (Putnam and Allshouse 1999), Americans annually consume 2.2 pounds of tree nuts, 5.8 pounds of peanuts, and 23.1 pounds of corn products. Assuming these quantities of foods were contaminated at the action level, the allowable daily dietary intake of total aflatoxins (A_T) B_1, B_2, G_1, and G_2 can be calculated as:
Tree nuts + corn products:

$$\begin{aligned} A_T/\text{year} &= (2.2 \text{ lbs nuts} + 23.1 \text{ lbs corn}) \times 20 \text{ ng } A_T/\text{g} \\ &\quad \times 0.45 \text{ kg/lb} \times 10^3 \text{ g/kg} \\ &= 227.7 \times 10^3 \text{ ng} \end{aligned}$$

Peanuts:

$$\begin{aligned} A_T/\text{year} &= 5.8 \text{ lbs peanuts} \times 15 \text{ ng } A_T/\text{g} \\ &\quad \times 0.45 \text{ kg/lb} \times 10^3 \text{ g/kg} \\ &= 39.15 \times 10^3 \text{ ng} \end{aligned}$$

Allowable daily dietary dose (ng/kg) of total aflatoxins:

$$\begin{aligned} &\text{Daily } A_T/\text{kg} \\ &= \frac{(227.7 + 39.15) \times 10^3 \text{ ng } A_T/\text{year} \times 1 \text{ year}/365 \text{ days}}{78.1 \text{ kg body weight}} \\ &= 9.36 \text{ ng } A_T/\text{kg} \end{aligned}$$

Alternatively, male rats were exposed in a nose-only chamber to aerosolized (mass median aerodynamic diameter [MMAD] 0.2 ± 2.2 μm geometric standard deviation (GSD)) purified AFB_1 at an airborne concentration of 3.17 μg/L for 20, 60, or 120 min (Jakab et al. 1994). Rats were killed 3 days post exposure for recovery of alveolar macrophages by lavage. Phagocytosis of sheep red blood cells was reduced in a time-dependent manner, significantly so in rats exposed for 60 and 120 min. Based on the minute ventilation for rats and assuming 15% fractional lung deposition for the 0.2-μm MMAD particle size range, these authors estimated that the pulmonary dose rate was 0.28 μg AFB_1/kg/min, equivalent to a 20-min dose of 5.6 μg/kg, which was used in this report as a comparison dose for AFB_1.

Satratoxins G and H

S. chartarum spores were administered intranasally to mice using two strains that contained either no detectable satratoxins ("nontoxic" s. 29) or 4×10^{-8} μg SG/spore and 10×10^{-8} μg SH/spore ("toxic" s. 72). A single administration of 10^6 s. 72 spores (4.7×10^7 spores/kg) killed two of four mice within 24 h and produced severe pulmonary inflammation and hemorrhage in the survivors (Nikulin et al. 1996). Subsequently, the same investigators administered intranasal doses of 10^3 and 10^5 spores (4.6×10^4 and 4.6×10^6 spores/kg) twice weekly for 3 weeks (Nikulin et al. 1997). No clinical signs were noted in any treated group, and there were no significant body weight changes. Necropsy at the end of treatment revealed hemorrhagic alveolar exudate and severe inflammatory responses in the lungs of mice treated six times with 4.6×10^6 s. 72 spores/kg. Lung inflammation was comparable in mice receiving six doses either of 4.6×10^6 nontoxic s. 29 spores/kg or of 4.6×10^4 toxic s. 72 spores/kg; there was no hemorrhage in either group. Combining the per spore content of SG (4×10^{-8} in μg/spore) and SH (10×10^{-8} μg/spore) reported by Nikulin et al. (1997), total satratoxins in a single exposure to the lower dose of s. 72 spores can be calculated for comparison:

$$\begin{aligned} &\mu\text{g satratoxins/kg} \\ &= 4.6 \times 10^4 \text{ spores/kg} \times 14 \times 10^{-8} \text{ } \mu\text{g (SG + SH)/spore} \\ &= 6.4 \times 10^{-3} \text{ } \mu\text{g/kg} \end{aligned}$$

Fumitremorgens

Fumitremorgen A was isolated from cultures of *Aspergillus fumigatus* FRES (IFM 4482), dissolved in a 2:3 *N*,*N*-dimethylformamide–propylene glycol mixture, then diluted in saline prior to being administered intravenously to eight groups of nine male dd/ys mice (Yamazaki, Suzuki, and Kukita 1979). Single intravenous (IV) doses ranged from 0.06 to 0.36 mg fumitremorgen A/kg, and mortality in the 24-h observation period ranged from 0/9 at 0.06 mg/kg to 9/9 at 0.36 mg/kg. The lowest dose produced "a perceptible tremor in a few mice," and 0.06 mg fumitremorgen A/kg was used as the comparison level.

Trichoverrols A and B

Because we were unable to identify any toxicity studies done on mammals with these mycotoxins, we chose to compare them to T-2 toxin, a trichothecene mycotoxin produced by *Fusarium* species and purified for use as a biological warfare agent. The US Army Center for Health Promotion and Preventive Medicine (USACHPPM) has established "military exposure guidelines" (MEGs) for air and drinking water exposure to chemical warfare agents (USACHPPM 2002). There is no air MEG for T-2 toxin, but the drinking water MEG "for a continuous daily consumption of either 5 L/day or 15 L/day for up to 5 days that should not impair performance and is considered protective against significant noncancer effects" is 0.026 mg T-2/L assuming consumption of 5 L/day (or 0.0087 mg/L for 15 L/day). Under these conditions, the 1-day dose of T-2 toxin at the MEG would be:

$$\mu\text{g T-2/kg} = \frac{5\ \text{L/day} \times 0.026\ \text{mg T-2/L} \times 10^3\ \mu\text{g/mg}}{78.1\ \text{kg}} = 1.66\ \mu\text{g T-2/kg}$$

Very nearly the same comparison level can be derived from 10-min inhalation exposures of mice, rats, and guinea pigs to T-2 toxin (Creasia et al. 1987, 1990). Rats were the most sensitive of the species tested, with a 10-min LC_{50} of 20 mg/m^3 and a no-effect concentration of 1.0 mg/m^3. Synthetic nonexchangeable [^{3}H]T-2 toxin was used to determine the retention of inhaled T-2 toxin in both rats and guinea pigs (Creasia et al. 1990). Based on measured whole-body retention of ^{3}H immediately following a 10-min inhalation exposure of rats to 23 mg [^{3}H]T-2/m^3, these investigators estimated that the 10-min LC_{50} corresponded to an LD_{50} of 46 μg/kg. Applying the measured ratio of retained dose to exposure concentration, the dose of T-2 corresponding to the 10-min no-observed-effect level (NOEL) concentration of 1.0 mg/m^3 can be estimated:

$$\frac{\text{Retained NOEL dose } (\mu\text{g T-2/kg})}{1.0\ \text{mg T-2/m}^3\ \text{NOEL concentration}} = \frac{46\ \mu\text{g T-2/kg}}{23\ \text{mg T-2/m}^3}$$

$$\text{Retained NOEL dose} = \frac{46\ \mu\text{g T-2/kg} \times 1.0\ \text{mg T-2/m}^3}{23\ \text{mg T-2/m}^3} = 2.0\ \mu\text{g T-2/kg}$$

RESULTS

Maximal Possible Doses (Table 3)

Maximum μg/spore data shown in Table 1 were substituted into equation [2] to calculate the maximum dose (ng/kg) of mycotoxins that a 78.1-kg man might inhale in a 24-h continuous exposure to 200,000 spores/m^3. Results are shown in Table 3 and compared to effects levels summarized in Table 2.

DISCUSSION

This article reports an expansion of a previously published (Burge 1996) risk assessment approach to include additional mycotoxins and comparisons to effect levels in animals exposed in vivo. Although there were data on inhalation exposure to purified mycotoxins, no data were available that involved inhalation exposure to spores containing known concentrations of mycotoxins. Studies that employed a variety of other exposure

TABLE 3
Maximum possible adult male 24-hour doses of mycotoxins versus reported effects levels from laboratory data

Mycotoxin	Maximum inhalation dose (ng/kg day)	Effect dose (ng/kg day)[a]	Ratio of effect dose to maximum dose
Aflatoxin B_1	19	5.6×10^3	3.0×10^2
Aflatoxin B_2	1.4	5.6×10^3	4.0×10^3
Satratoxins G + H	5.4	6.4	1.2
Fumitremorgen B	3.5×10^{-2}	60×10^3	1.7×10^6
Fumitremorgen C	0.45	60×10^3	1.3×10^5
Verruculogen	8.6×10^{-2}	60×10^3	7.0×10^5
Fumitremorgen B + verruculogen	3.1	60×10^3	1.9×10^4
Trichoverrols A + B	5.8×10^{-2}	2.0×10^3	3.4×10^4

[a] See Table 2.

modalities provided data that permitted calculation of mycotoxin dose-effect relationships. However, the relevance to human inhalation exposure-response is uncertain for nonphysiologic dosing regimens (intranasal and intravenous dosing) and for ex vivo measures of uncertain relevance to health status (macrophage phagocytosis).

Limited data made it difficult to establish mycotoxin content in spores. Measured concentrations of mycotoxins in spores recovered from molds growing on building materials in an indoor environment would have been most directly relevant, but an extensive search of the peer-reviewed literature found no data of this type. Fungi isolated from an indoor environment and grown on building materials in the laboratory could have provided desirable surrogate data, but published reports of that kind did not permit calculation of per-spore mycotoxin concentrations. The published data that did provide per-spore mycotoxin concentrations employed laboratory cultures on defined media and therefore cannot be considered representative of mycotoxin production under conditions of growth on building materials in the indoor environment.

The model we used to estimate maximum possible doses of inhaled mycotoxins is conservative in multiple ways. Firstly, the assumptions of 100% retention of spores inhaled and 100% bioavailability of mycotoxins in spores is conservative as both retention and availability are likely to be less than 100%.

Secondly, continuous inhalation exposure to a concentration of 200,000 spores/m^3 for 24 h in a residential, office, or school environment is improbable. Both our experience and published air sampling data from water-damaged and moldy buildings indicate that concentrations of 200,000 spores/m^3 are possible but rarely found even in obviously moldy indoor environments. In a study of 62 homes in Great Britain (Hunter et al. 1988) that were sampled because there had been complaints of dampness or mold growth, the highest count reported was 449,800 colony-forming units (cfu)/m^3 and in one home a count of 17,900 *S. chartarum* cfu/m^3 was recorded[2] (the authors noted that some sampling coincided with construction work in the homes, but they did not distinguish those samples from others). Overall summary statistics were not provided, but interquartile ranges (and number of samples taken) in rooms with visible mold growth were bedrooms, 406 to 5615 cfu/m^3 (163); living rooms, 1165 to 4944 cfu/m^3 (61); kitchens, 1107 to 5862 cfu/m^3 (41); bathrooms, 388 to 5615 cfu/m^3 (13). If there were health complaints in any of the homes sampled, none were mentioned. In the largest published collection of indoor sampling data in the United States, representing results from 9619 samples collected in 1717 buildings, the maximum indoor spore count was "$>$10,000 cfu/m^3" (95th percentile 1300 cfu/m^3) (Shelton et al. 2002). The 200,000 spores/m^3 used for modeling is more representative of conditions reported inside an enclosed containment structure where the total spore trap count was 223,000 spores and hyphal fragments/m^3 during removal of a wall that was extensively colonized (over 100 square feet) with visible mold (Morey and Sawyer 2001).

Finally, the model is highly conservative in the amounts of mycotoxin assumed to be present in spores. It is well-documented that even for fungal species and strains capable of producing mycotoxins, their production is highly variable and dependent on multiple factors that include environmental conditions and substrate (Jarvis et al. 1998; Land et al. 1994; Nikulin et al. 1994; Rao 2001; Ren, Ahearn, and Crow 1999; Sorenson et al. 1987; Tuomi et al. 2000). Furthermore, competitive interactions may either decrease or increase the amount of mycotoxin produced (Dalcero et al. 1989; Picco et al. 1999). None of the maximal mycotoxin concentrations used for modeling were derived from molds growing on construction materials. Rather, all maximal mycotoxin concentrations were derived from fungi grown in pure culture on defined media in the laboratory under conditions designed to optimize mycotoxin production. When toxigenic fungi (including the same strains that were modeled) were grown on building materials, they produced much reduced or nondetectable levels of mycotoxin relative to the levels used for modeling (Nikulin et al. 1994; Ren, Ahearn, and Crow 1999). Furthermore, with a single exception (Land et al. 1994), the mycotoxin data modeled were derived not from environmental isolates but from laboratory reference strains known to produce significant amounts of mycotoxins. Thus, the maximal concentrations used for modeling must be regarded as an overestimate of the mycotoxin content of molds growing in water-damaged buildings.

To provide context for the maximum possible inhalation doses, conservative comparison doses were selected. The comparisons for fumitremorgen (Yamazaki, Suzuki, and Kukita 1979) and the satratoxins (Nikulin et al. 1997) are derived from nonphysiologic exposures (IV and intranasal instillation), whereas the comparison for AFB_1 (Jakab et al. 1994) is derived from endpoints that have unknown relevance, if any, to the in vivo health of the exposed animals. The 'effects' levels chosen for comparison purposes therefore may not be predictive of discernable health effects in humans exposed to similar doses of these mycotoxins by inhalation.

Although comparison levels here must be regarded as lowest-observed effect levels (LOEL)s rather than NOELs, the ratio of "effect dose" to "maximum dose" is similar to the "margin of exposure" (MOE) as used by the US EPA, which is the NOEL divided by the level of human exposure. When the MOE is greater than 100, the level of concern is low. Under the conditions of this model, the maximum inhalation dose (Table 3) was lower than the comparison dose by a factor of 10^2 (AFB_1) to 10^6 (fumitremorgen B) for all mycotoxins except satratoxins G and H. Thus, the model suggests that, even for an extraordinary exposure of 200,000 spores/m^3, there is a wide margin of safety

[2]"Colony-forming units" represent those collected spores that had established a visible colony at the time of enumeration. Many collected spores are not viable, and some viable spores may fail to establish visible colonies due to slow growth, unfavorable medium, competition, etc. This model was based on "spore counts" on the presumption that mycotoxin content does not vary among viable and nonviable or culturable and nonculturable spores.

for all the mycotoxins modeled except satratoxins G and H. For the satratoxins, and specifically for the highly toxic s. 72 *S. chartarum* spores on which the model was based, determining the appropriate level of concern for an exposure of 200,000 spores/m^3 would require a more detailed evaluation of the degree of conservatism in the model calculations.

For the aflatoxins, ingestion data may be relevant for deriving a comparison dose as no significant difference was seen in the excretion and distribution of radioactivity after either oral or intraperitoneal administration of AFB_1 in male rats, implying that absorption after oral exposure was complete (Wogan, Edwards, and Shank 1967). The calculated maximal inhalation dose of AFB_1 (19 ng/kg day) is higher than the estimated daily dose of total aflatoxins (9.4 ng/kg day) that could be consumed if foods were contaminated at the applicable action levels. However, the aflatoxin dietary action levels are intended to prevent chronic health effects associated with life-long ingestion of aflatoxin-contaminated foods. In addition, aflatoxins have not been identified from molds growing in indoor environments. Notably, when *A. flavus* was grown on building materials in the laboratory, no aflatoxin production could be detected (Ren, Ahearn, and Crow 1999). If the AFB_1 content determined in that study in an environmental isolate grown on a defined medium (3.3×10^{-8} μg/spore) is used in equation [2] (rather than the data used from a laboratory reference strain), the calculated maximum daily dose is 1.3 ng/kg. At dietary action levels, the estimated daily dose of total aflatoxins (9.4 ng/kg day) is 7.2 times higher, and the inhalation NOEL level from rats (Table 2) is 4.3×10^3 times higher.

With respect to satratoxins G and H, the highest published airborne concentrations of *S. chartarum* that we located were 7500 spores/m^3 in a semiagricultural environment (Dill, Trautmann, and Szewzyk 1997), and 17,900 cfu/m^3 in a water-damaged dwelling in Great Britain (Hunter et al. 1988). The authors were not specific, but the latter count probably was determined during renovation of a moldy home. In the semiagricultural environment, workers were potting plants into moldy decomposable pots. These workers experienced severe dermatitis of the fingertips and investigation revealed *Acremonium, Chaetomium, Trichoderma,* and *S. chartarum,* among others, on the pots. When pots were being moved, *S. chartarum* spores were measured at airborne concentrations of 7500 spores/m^3. Obvious dermatitis experienced by these workers suggests that mycotoxins were present, whereas the lack of reported pulmonary or systemic effects suggest that airborne exposures were not sufficient to produce toxicity via inhalation. Under normal conditions, with no active disturbance of the colony, few *S. chartarum* spores are aerosolized (Rao 2001; Wilkins et al. 1998). In 9619 samples collected in 1717 buildings, *S. chartaum* was identified in 6% of buildings, and in those buildings the median concentration was 12 cfu/m^3 (95% confidence interval [C.I.] 12 to 118 cfu/m^3); *S. chartarum* was identified in the outdoor air of 1% of the buildings studied, with a median concentration of 12 cfu/m^3 (95% C.I. 4 to 318 cfu/m^3) (Shelton et al. 2002). These data suggest that the 200,000 spores/m^3 default in the model incorporates at least a 10^3 margin of safety for *S. chartarum* exposures.

Exposure conditions in agricultural settings have been associated with acute health effects when airborne bioaerosol levels were in the range of 10^9 to 10^{10} spores/m^3 (Malmberg, Rask-Andersen, and Rosenhall 1993). Those exposures were greatly in excess of the 2×10^5 spores/m^3 employed in our model. The model suggests that 2×10^5 *S. chartarum* spores/m^3 would cause acute irritation if they contained satratoxins G and H at the levels modeled, but that those spores likely would not produce a systemic mycotoxicosis. For all of the other mycotoxins modeled, there is a wide margin of safety even if spores containing the modeled amounts of mycotoxin are present at a concentration of 2×10^5 spores/m^3.

CONCLUSIONS

The current model illustrates that delivery of mycotoxins via inhalation of mold spores is inefficient and suggests that mycotoxin intoxication does not follow inhalational mold spore exposure in indoor environments due to the requirement for extremely high airborne spore levels and extended periods of exposure. The comparison data indicate that it is highly unlikely that the dose of mycotoxin received in an indoor home, office, or school environment could approach levels that would produce an acute toxic response, even under the extreme conditions modeled. Under the exposure conditions commonly encountered in a visibly moldy indoor environment, the potential for inhaling a toxic dose of mycotoxins is remote.

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