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A Quantitative Risk Assessment for *Stachybotrys chartarum*-Toxic Mold

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Abstract

The inhalation exposure to *Stachybotrys chartarum* (SC) has been playing a critical role in public health domain due to its ability to produce toxins (trichothecene and other mycotoxins), that are implicated in the cases of acute idiopathic pulmonary hemorrhages (AIPH) in infants. Despite multiple studies reporting an association of exposure to airborne mold with negative human health effects such as respiratory, immunological, and hematological diseases, there currently are no regulatory guidelines for quantifying the airborne exposure threat associated with SC spores/toxins in indoor environments. The primary goal for this study was to use risk assessment framework as a mechanism to quantify the potential risk of death associated with known concentrations of S. *chartarum* spores and toxins. Additionally, an attempt was made to provide a benchmark dose level above which an adverse effect may occur in human infants. To this end, best-fit dose-response models were generated by using published animal studies, followed by stochastic risk assessment to predict the likelihood of death in infants from AIPH. By using 10% as the benchmark response level, permissible exposure limits were obtained as 413 SC-spores/m^3 or 2.66 x 10^{-04} mg-toxin/m^3. The predicted risk of death in infants for acute 24-hour exposure to toxins ranged from 1.0 x 10^{-11} to 3.0 x 10^{-06}. Sensitivity analysis was conducted using Monte Carlo simulations to identify factors that are highly correlated with risk estimates. This study suggests that S. *chartarum* exposure via inhalation in residential conditions may pose a risk for AIPH resulting in mortality in human infants, but a conclusive epidemiological study is needed to validate risk estimates.

Keywords: *Stachybotrys chartarum*; Trichothecene; Risk Assessment; Exposure Assessmnet; Dose-Response modeling; Acute Idiopathic pulmonary hemorrhage (AIPH)

Abbreviations: SC: *Stachybotrys Chartarum*; AIPH: Acute Idiopathic Pulmonary Hemorrhages; PEL: Permissible Exposure Limits; QRA: Quantitative Risk Assessment; BAL: Bronchoalveolar Lavage; EPA: Environmental Protection Agency; BMD: Benchmark Dose; NOAEL/LOAEL: No Observable Adverse Effect Limit or Lowest Observed Adverse Effect Limit; BMDL: Lower Benchmark Dose; BMR: Benchmark Risk; HED: Human Equivalent Dose; LTA: Luciferase Translation Assay ;

ELISA: Enzyme Linked Immunosorbent Assay; HPLC: High Performance Liquid Chromatography; SGE: Satratoxin G Equivalents; MTM: Macrocyclic Trichothecene Mycotoxin; MLE: Maximum Likelihood Estimation; KS: Kolmogorov-Smirnov; BA: Bioavailability; CI: Confidence Interval; PPE: Personal Protective Equipment's; BALF: Bronchoalveolar Lavage Fluid; MMWR: Morbidity and Mortality Weekly Report.

Introduction

In recent years, public attention on human health issues has focused on health risks associated with fungi in the home environment [1-3]. Inhalation exposures to fungi can lead to respiratory, neurological, and dermatological diseases such as asthma, hypersensitivity pneumonitis, upper respiratory symptoms, coughing and wheezing [4]. Fatal responses to fungi inhalation such as pulmonary edema, hemorrhage, and lethargy have been widely documented in animal studies, but lack epidemiological evidence [5]. Despite documentation of associated health issues, there is currently no federal standard for exposure limits to mold within residential households [6,7]. Within the United States, a drastic rise in multimillion dollar mold lawsuits has become a major issue for home owners and building managers demanding a framework to assess the health risks that dampness and mold poses to humans in indoor environment [8,9]. Of primary concern is the provision requiring the establishment of minimum levels of exposure to mold, as well as the uncertainty inherent in these minimum levels [10].

Of the estimated 100,000 existing species of mold, only a few thousand can currently be identified and only about 50 have been classified as "toxic molds" by the Center for Disease Control and Prevention CDC [11]. Out of these known toxic-molds, *Stachybotrys chartarum* came into public attention in the 1990s when a cluster of infant pulmonary hemosiderosis and hemorrhage was identified in Cleveland, Ohio [12,13]. Although water-damaged indoor environment was identified as a major risk factor and probable cause for mold growth, toxin exposure was inconclusive for causing AIPH in infants due to low measured concentration of spores [14,15]. Due to *Stachybotrys chartarum*'s ability to produce a chemically diverse group of potentially toxic metabolites known as "mycotoxins", it is also often called the "fatal fungus" [16,13].

In spite of detailed toxicity data on mycotoxins, the health risks from inhalation exposure to S. *chartarum* remains poorly defined. S. *chartarum* can produce two chemotypes, macrocyclic trichothecenes mycotoxins and atranones, but the level of toxin production is highly variable due to environmental factors [17,18,13]. Traditional toxicologic and epidemiologic study designs examining airborne exposure to black mold were inconsistent and unreliable to enumerate indoor exposure concentrations; which lead to uncertainty on the extent of human toxicity to spore and toxin exposure [19,17]. Multiple animal studies have documented pulmonary hemorrhage in infantile rats through higher concentrations of erythrocytes and hemosiderin in lung samples. It is believed that trichothecene toxins depress the collagen synthesis in lung tissue and therefore infantile lungs are more at risk for inflammation and injury due to the rapidly dividing cells. It is not widely understood why some strains of mycotoxins produce fatal responses while other subjects only experience inflammatory responses [14,20,21].

Given the controversies surrounding the health risk characterization of molds, development of a federal unified system of mold-regulations has been a big challenge. In 2001, California was the first state to pass comprehensive mold legislation. California's Toxic Mold Protection Act mandated that the California Department of Health Services determine the 1) feasibility of setting health-based Permissible Exposure Limits (PELs) for mold and 2) develop standards of mold assessments, in indoor environments by gathering a task force of health and medical experts, community officers, and government representatives [10]. Following California's law as a model, few other states have also passed legislation setting forth guidelines and safety standards for mold exposure. However, due to lack of exposure-response data and fungal-diversity in indoor environment, to date no clear state or federal policy has been issued on PELs for airborne concentrations of mold, or mold spores and on mold assessment standards [22].

A detailed analysis of animal studies can provide a better insight on the toxicity of spores to human populations through the development of a mathematical model. The development of a mathematical relationship between exposure indicators and health outcomes could lead to a scientific assessment that characterizes the associated health burden in human populations. It is imperative to investigate the pathological potential of this fungus and its toxins in the most sensitive human population, i.e. infants, due to the great number of animal studies reporting mortality as the disease endpoint. Thus, in this study a stochastic risk assessment approach was applied by mathematically combining the available toxicity and exposure information on *S. chartarum* mold

to predict the environmental levels that are unlikely to be of health concern with respect to both spores and toxins. The overall objective of this study was to develop a risk assessment framework that would serve as a tool to evaluate the extent in which the presence of *S. chartarum* spores/toxins produce inflammatory responses and mortality in infant populations. A secondary objective of this work was to provide a benchmark dose level above which an adverse effect may occur in human infants. Human infants were selected as the subject population because of their susceptibility to respiratory illnesses due to immature lung development.

Methodology

The approach of Quantitative Risk Assessment (QRA) was adopted to determine the potential risk for AIPH in human infants from inhalation exposure to *S. chartarum*. Intensive literature review was conducted to collect relevant data on animal studies and indoor exposure factors associated to AIPH in infants. The following sections outline the sources for dose-response and exposure assessment, and description of statistical approach used to probabilistically quantify the collected information.

Data sources

Dose Response Data:

Animal Studies: Many documented studies have monitored the hematological parameters and degrees of lung inflammation that resulted from introduction of S. *chartarum* spores and mycotoxins [23-25,20,21]. However, the data from a large portion of these studies could not be used in the dose-response model because negative adverse effects (such as illness) from dosing trials were not recorded. The two primary studies used to quantify the potential level of illness in human infants from S. *chartarum* spores/toxins were Fairhurst et al. 1987 and Yike 2001 [26,27]. The specific inclusion criteria for a study to be considered in the dose-response modeling consisted of a clinical end-point of death or pulmonary hemorrhage following exposure, and a minimum of three different dose levels in the given experiment. Table 1 summarizes the studies used to elicit dose-response data for the QRA.

Author/Year	Title	Host	Dosing	Detection Mtds	End point
Fairhurst et al. 1987	Acute Toxicity of T2 Toxin in Rats, Mice, Guinea Pigs and Pigeons	Female rats, (180-220g)	0.5 ml/ kg -Intratracheally in two different suspensions (Final dose in mg/kg) in saline suspension	direct toxin suspension was given (no method required)	death
Yike 2001	Infant animal model of pulmonary mycotoxicosis induced by *Stachybotrys chartarum*	4 day old rat pups	Non-viable spores of SC strain JS5817 in saline from Cleveland, Ohio case injected Intratracheally.	Spores in suspension were counted	death

Table 1: Summary of the studies used to elicit dose-response data for the S. *chartarum* scenario.

Fairhurst (1987) dosed female rats with T2 toxins via intratracheal administration with a saline solution. Yike (2001) dosed four-day-old rat pups with non-viable spores of S. *chartarum,* which were obtained from the Cleveland, Ohio case. The endpoint in all cases was death with evidence of pulmonary hemorrhage. While multiple studies involving bronchoalveolar lavage (BAL) analysis were reviewed, they were not used for the dose-response modeling due to the lack of data to provide a distinct positive or negative response.

Human Equivalence Approach: Environmental Protection Agency (EPA) guidelines cite the Benchmark Dose (BMD) approach as more quantitative than the current No Observable Adverse Effect Limit or Lowest Observed Adverse Effect Limit (NOAEL/LOAEL) process for non-cancer health effects. Therefore, in our assessment of determining a threshold of exposure that does no harm, we used the Lower Benchmark Dose (BMDL) approach. To establish the BMDL, confidence limits were used as outlined in the EPA training and methodology manual. The default Benchmark Risk (BMR) is an excess risk of ten percent, therefore for the examined cases a corresponding 0.1 risk value was used. Two uncertainty factors were considered during the QRA –the animal-to-human equivalency and the uncertainty

due to questionable data sets. Since our data represents various strains and animals, this uncertainty factor was deemed appropriate; the corresponding dose was therefore divided by two orders of magnitude to give the BMD.

Finally, application of the correction factor for weight and surface area provided the Human Equivalent Dose (HED) [28].

$$HED = dose_{animal} \times \left(\frac{\text{animal } K_m}{\text{human } K_m}\right)$$

K_m is the ratio of body surface area to weight of the animal and human, respectively. To convert the dose in terms of concentration, this figure was divided by the default values for infant breathing rate and body weight.

Exposure Data

The exposure dose evaluation in human infant lungs could be done either by using spores or mycotoxins as indicators of exposure. Although there are challenges with the measuring methods for both indicators [29,30,31], studies using detection methods that are widely tested for accuracy such as chromatography, immunoassays, and protein translation inhibition assays were reviewed to characterize toxin concentration. Similarly, due to uncertainties associated with the quantification of viable spores [32,27], studies reporting non-viable spore concentrations in indoor environments were reviewed. Table 2 outlines the data sources and reported indicator concentrations, analyzed for the QRA.

Toxins quantification in SC spores						
Study	**strain/spore**	**Viability[a]**	**Method[b]**	**Reported Avg. indicator/media[c]**	**toxin/spore-emperical cal[d]**	**Data points**
Yike et al. (1999; 2001)	JS5817	N	LTA	12,391pg-SGE/m^3 - air	1pg SGE/spore	10
				6500 pg-T2/m^3-air	0.5pg- T2/spore	10
Brasel et al. (2005)	JS5818	N	ELISA	250 pg/m3 (SG,SH, RA, VA)-air	0.2pg Trichothece/spore	11
Sorenson et al. (1987)	ATCC-62752, 60	not clear	HPLC	19400 pg (SG, SH, TriAB)/mg dust[e]	0.005 pg Trichothece/spore[f]	7
Brasel et al. (2005)	spore	NA[g]	BH2-RFCA	4,944 spore/m^3 -air	NA	11

Table 2: Toxin Concentration in indoor environment, associated with *S. chartarum*.

[a]N, stands for non-viable spores
[b]Toxin detection methods: Luciferase Translation Assay (LTA), enzyme linked immunosorbent assay (ELISA), High Performance Liquid Chromatography (HPLC).
[c]Toxin concentrations reported in terms of -SGE (Satratoxin G equivalents), Average Trichothecene equivalents (Sat-G, Sat-H, Rori-L, Trichoverols-A,B)
[d]Toxin per spore were derived by empirical calculations based on average trichothecene equivalents for the entire collected sample, collection time, and flow rate of spore collection sampler.
[e]Toxin concentration including (Satratoxins and Trichoverol A and B) give in per unit weight of dust particles
[f]Concentrations from Per unit weight of dust were converted to per spore, by applying the approach discussed by (Kelman and others 2004) [33]. In short, spores mass of 2.6 $x10^{-7}$ mg/spore was applied to dust samples, which were described to have 85% spores in their composition.
[g] not applicable

Brasel and co-authors measured non-viable indoor spore concentration in Texas, on eight water-damaged buildings using Olympus BH2-RFCA optical light microscopes [29]. As indicated in Table 2, the concentration ranged from 226 to 16,968 spores/m^3. This was the maximum reported *S. chartarum* spore concentration found in current literature and the only study quantifying non-viable spores. The same study determined Trichothecene values in the air using a macrocyclic trichothecene mycotoxin (MTM)-specific enzyme linked immunosorbent assay (ELISA). This assay has previously demonstrated high specificity to MTMs with no cross-reactivity with other common indoor fungi [34,35]. The two other studies considered for QRA used the Luciferase Translation Assay (LTA) and High Performance Liquid Chromatography (HPLC) methods for toxin quantification from *S. chartarum* exposures. The LTA method is based on inhibition of protein synthesis and was tested for the detection of trichothecene mycotoxins by Yike and other researchers [31]. Sorenson

and co-authors used the HPLC method to investigate respirable sized aerosolized trichothecene mycotoxins [30].

Data Analysis

Dose Response Modeling: The threshold models were used in lieu of the standard Exponential and beta-Poisson models found in microbial risk assessment because exposure to non-viable spores and toxins are classified as chemical exposures. This is an important distinction as the Poisson distribution can no longer be assumed for the scenarios being investigated in this QRA. As such, the equations for the empirical Log-probit, Weibull, and Log-logistic models appear below:

- Log-probit:
$$p(d) = \Phi\left(\frac{1}{q_2}\ln\frac{1}{q_1}\right) \text{ where } \Phi(y) = \frac{1}{\sqrt{2\pi}}\int_{-\infty}^{y}\exp\left(-\frac{x^2}{2}\right)dx$$
- Weibull: $p(d) = 1 - \exp(-q_1 d^{q_2})$
- Log-logistic: $p(d) = \frac{1}{1+\exp[q_1 - q_2\ln(d)]}$

where q_1 and q_2 are parameter values for the dose-response models and p(d) is the probability of illness/death at a given dose d. Entire statistical programming for dose-response assessment was performed in R software. The model parameters were determined by minimizing deviances of the threshold models using approaches of maximum likelihood estimation (MLE). To test which model provided a statistically acceptable fit, the log likelihood ratio (deviances) was compared with the critical chi-squared distribution at a 95% confidence level with appropriate degree of freedom.

Human Exposure Assessment: Stochastic modeling approach was used to represent the indoor concentrations of toxins and spores in order to capture the uncertainty present in environmental sampling. The best distribution to model the spore and toxin concentration was determined via the Kolmogorov-Smirnov (KS) goodness-of-fit test in Crystal Ball (Statistical software) by using maximum likelihood estimation. Where enough information was not available for stochastic modeling (e.g. toxin/spore), a Log-normal distribution fit was assumed to represent the probability distribution function for that parameter. The standard EPA default values for inhalation from the exposure factor handbook were applied to obtain the distribution of breathing rate and body weight of infants under one year of age. The final distributional fits used for the QRA from acute exposure to spores and associated toxins are summarized in Table 3.

Input variable	Distribution[a]	Unit	Source
C(toxin conc'n)	LN(10178,50488)	pg/m³-air	Brasel et al. (2005); Yike et al. (1999)
	LN(1,0.5)	pg/spore	Sorenson et al. (1987)
N (spore conc'n)	Beta(226, 983, 5984, 17000)	spores/m³-air	Brasel et al. (2005)
BR (Breathing Rate)	LN(700, 1.46)	Lts/kg-day	Amy Arcus (2007)
BW (Body weight)	LN(9.4,1.19)	Kg	Finley et al (1994)

Table 3: Input distributional fits for inhalation exposure estimates in human infants.

[a] Best fit distributions: LN- Log Normal (Mean, std dev.), Beta (Min, 25%'ile, 75%'ile, Max)

Potential Human Dose calculation: The dose of mycotoxin and spore inhaled by an infant in a one day (24h) acute exposure scenario was calculated as follows:

i) $$\text{Dose}\left(\frac{\text{spores}}{\text{day}}\right) = N_{\frac{\text{spores}}{\text{m3}}}.\ BR_{\frac{\text{m3}}{\text{kg}}-\text{day}}.\ BW_{\text{kg}}.\ FR.\ BA$$

ii) $$\text{Dose}\left(\frac{\text{pg}}{\text{day}}\right) = Ca_{\frac{\text{pg}}{\text{m3}}}.\ BR_{\frac{\text{m3}}{\text{kg}}-\text{day}}.\ BW_{\text{kg}}.\ FR.\ BA$$

where N is the number of spores/m³ of air, BR is the normalized breathing rate (m³/kg-day), BW is infant body weight (kg), C is mycotoxin concentration in spores (pg/spore), Ca is mycotoxin concentration in air (pg/m³), FR is the fraction of inhaled spores deposited in the lungs,

and BA is fraction of mycotoxin which is released and biologically available. As an upper limit on retained dose we assumed the mycotoxin bioavailability (BA) as 100%; it is a function of spore size and typically bioavailability increases with decrease in the size [4,30]. The fraction of aerosols deposited (FR) was assigned a uniform distribution with a range from 30% to 50%. This number is based on a recent study by Lee et al., who developed mathematical models to predict the deposition fractions in respiratory tracts of infant rat models and indicated that the maximum fraction observed to be deposited in most animal studies is 0.3 [36]. Furthermore, as a conservative measure, in our dose model we assumed this deposition fraction to range from 30% to 50% and applied a uniform distribution to this variable. Finally, the daily dose for acute exposure scenario in infants were obtained by a Monte Carlo analysis in which values were randomly drawn from the probability distribution functions of concentration, breathing rate, deposition fraction and body weight as defined in Table 3.

Results and Discussions

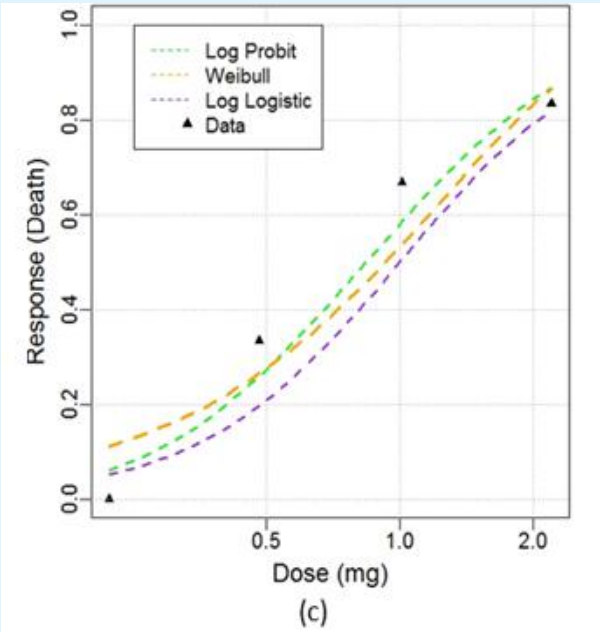
Benchmark Dose and Model Development

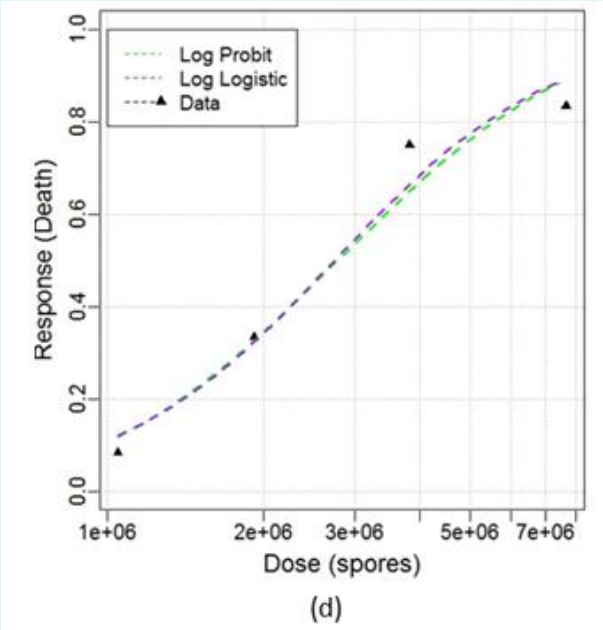
As discussed in the Dose-response method section, the BMD approach was used to provide a benchmark dose level above which an adverse effect may occur in human infants. The MLE outputs of the dose-response analysis for each of the studies (listed in Table 1) are summarized in Table 4 and the corresponding curves with the bootstrap result of the best fit-model are shown in Figure 1. In all three models the log likelihood ratio (deviance) was found to be less than the critical chi square values; this suggests that each model provides an accurate representation of the dose response relationship of the data. We further investigated the slope of the models in the lower dose range and selected the model that resulted in most conservative estimate for a low dose extrapolation in each study category (Table 4). The Fairhurst rat model bootstrap did not converge, therefore the graph was omitted. The Yike study bootstrap was generated from the Log Logistic curve.

Study (Critical Chi-sq	**Fair 1987 mix rats (CRI CHI = 5.99)**			**Fair 1987 saline rats (CRI CHI = 3.84)**			**Yike 2001 infant rat pups (CRI CHI = 5.99)**		
Model	**Deviance**	**Parameters**	**SEM**	**Deviance**	**Parameters**	**SEM**	**Deviance**	**Parameters**	**SEM**
wei	**1.03**	**0.75**	**1.54**	2.4E-14	806129.8	---	---	---	---
		1.23	**1.58**		29.9641	---		---	---
Log probit	0.60	0.86	1.55	---	---	---	1.06	0.83	3.63
		0.84	1.44		---	---		2676445	3.27
Log logistic	0.96	0	---	---	---	---	**0.98**	**30.57**	**3.76**
		1.92	1.62		---	---		**2.05**	**3.76**

Table 4: MLE Dose-Response Statistical Outputs[a]

[a] Bold are the final dose-response models that were considered for QRA





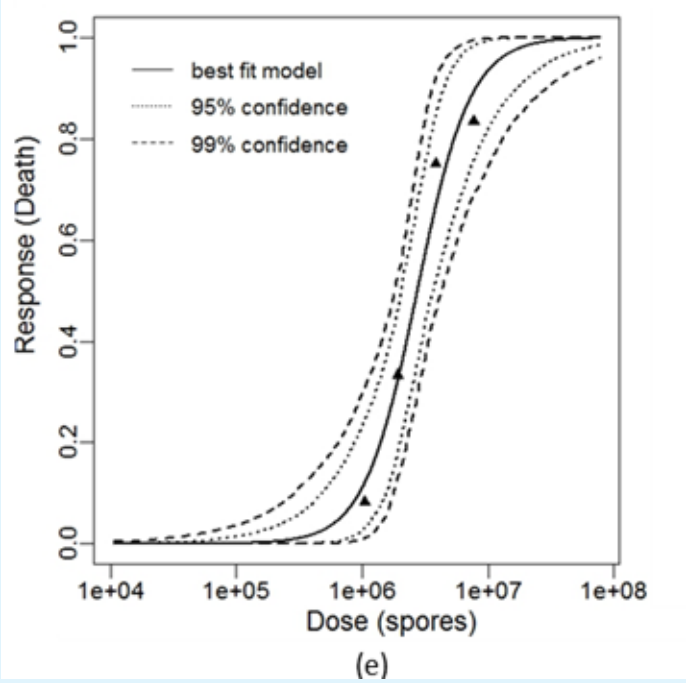


Figure 1: (c): Log-Probit, Weibull, Log Logistic models for Fairhurst Rat Model. (d): Log-Probit and Log Logistic models for Yike Rat Model. (e): Log logistic model with line of best fit, 95% confidence and 99% confidence, for Yike Rat Model.

The Weibull model was considered the most conservative model for the Fairhurst study where toxins were used as dose. On the other hand, the Yike study used spores as the dosing unit and the Log logistic model proved as a more conservative model than Weibull (Table 4). Based on the dosing units in the animal studies, two

risk models were proposed – Model 1 with toxin as dosing unit and Model 2 with spores as dosing unit (Table 5). Using these dose response models, HED in terms of both toxins and spores resulting in less than 0.1 risk of infant mortality due to AIPH was estimated as 2.66 x10^{-4}mg/m^3 and 413spores/m^3 (Table 6).

DR-Model (Dosing Unit)	Best fit model eq'n[a] (Animal species/Route)	Parameter[b]
Model- 1(pg-T2)	1-exp(-q1*d^{q2})	q1 = 0.76, SEM = 1.54;
	(Rats/Intratracheal)	q2 = 1.23, SEM = 1.58
Model- 2 (spores)	1/(1+exp(q1-q2*ln(d)))	q1 = Max Ext. (28.8, 8.71);
	(Infant Rat/Intratracheal)	q2 = Max Ext. (1.94, 0.59)

Table 5: Risk models for projections of infant death probabilities.

[a]The best fit models as Weibull when directly toxins were given to animals (Model 1) and Log logistic when spores were given to animals (Model 2).
[b]The best fit model's parameters obtained from 4000 bootstrap iterations, N=Normal distribution(mean, std dev.), Max Ext.=Maximum Extreme distribution (likeliest,scale).

Risk Model	Corresponding Dose (at 0.1 risk level)	Benchmark dose (BMD) (adj. UF's)[a]	Human Equivalent Dose (HED)	Concentration (Environmental concentrations)	
				Value	Units
Model 1	0.61 mg	6.1E-03 mg	1.75E-03 mg	2.66E-04	mg/m^3
Model 2	9.48E+05 spores	9.48E+03 spores	2.72E+03 spores	4.13E+02	spores/m^3

Table 6: Summary of MLE Dose-Response Output and Calculations.

[a]UF's are the uncertainty factors

Human Risk projections

The above mentioned dose-response models were developed by analyzing the animal studies that used either T2 toxins or spore concentrations as their dosing unit. The structural and functional differences between the physiology and respiratory system of rodents and primates makes it challenging to extrapolate human risk projections from rodent studies. A recent study conducted by Carey, Plopper et al. discussed the role of acute exposure to Satratoxin-G and *Stachybotrys* in the nasal and neurotoxicity of young adult rhesus monkeys [37]. They concluded that intranasal exposure to Satratoxin-G in mice showed morphologically similar nasal lesions to those observed in monkeys at comparable doses. Based on this knowledge and the similarities in structure and function of the respiratory tracts between monkeys and humans, we directly applied the rodent model for human risk assessment.

Following 10,000 Monte Carlo iterations, the projected retained dose from each of the dose equations (eq. i and eq. ii) was substituted into the appropriate dose response models to predict the probability of an infant dying from the acute exposure to *Stachybotrys* fungi. The results from each of the model, including the average and 95% confidence interval (CI) value, are shown in Table 7.

DR model (animal, dosing unit)	Average risk of Death in infants	95% CI
Model – 1 (Rats, Toxins)	$3.24e^{-06}$	$6.4e^{-09}$ – $9.9e^{-06}$
Model – 2(Infant Rat pups, Spores)	$1.26e^{-11}$	$6.8e^{-13}$ – $3.8e^{-11}$

Table 7: Average risk of death in infants per acute 24hrs of exposure.

Significantly high risk of death was projected when toxins were considered as the indicator of exposure (Model 1 versus Model 2, Table 7). It should be noted that there are significant intra-species differences in mice models and hence the mold susceptibility is likely to differ across the species [38,39]. Human relevance to these model predictions can be further tested if quality epidemiological studies can be found regarding AIPH in infants.

On the other hand, when spores were used as exposure indicator (with an approximate maximum spore concentration of 17,000 spores/m^3, Brasel et al. 2005) the estimated risk was significantly low approximately 1.26x10^{-11}, Table 7. This result of low infant mortality risk

based on ambient spore quantification is similar to findings from other researchers where, it has been found that an acute inhalation exposure resulting in immediate adverse health effects would require a total spore concentration between the orders of 10^5 to 10^9 spores per cubic meter [40]. Such high concentrations are improbable for residential and commercial indoor ambient air and inconsistent with reported airborne concentrations [40]. It is important to note here that the presence of toxins have been reported in particles smaller than spore conidia (such as dust fragments etc.), requiring more efficient sampling protocols to capture the full spectrum of conidia, fragments and dust particles that hold the potential for SC exposure [34]. Thus, one explanation for observed discrepancy between the two risk estimates could be significantly low representation of spore counts as compared to aerosolized toxins when collecting the environmental samples. Additional uncertainties associated with spore quantification, such as potency of viable spore versus non-viable spores, further challenge the application of spore based dose-response models.

Multiple approaches have been used in past to evaluate the risk associated with S. *chartarum* spore inhalation. One approach cited in literature included calculating the maximum possible dose of mycotoxins that could be inhaled in 24h of continuous exposure to high concentration of mold spores containing the maximum reported concentration of toxins. The maximum possible dose was then compared to the NOAEL dose measured in animal studies [33]. Similarly, Hardin and co-authors estimated airborne concentrations representing human doses comparable to animal no-effect dose levels and proposed human comparable dose of as high as 2.1×10^6 SC spores/m^3 for infants [15]. Such findings requiring significantly high spore concentrations to cause an adverse effect in human health has led policy makers to believe that exposure to most mold contaminated indoor environments would not result in measurable detrimental health effects [41]. Although another implication of such discrepancy between spore v/s toxin risk threat may suggest the use of personal protective equipment's (PPE) in occupational settings as insufficient as most of the PPE (masks) focuses on spore removal only.

Sensitivity Analysis

The risk estimates derived through two dose-response models were further analyzed to identify key input parameters causing maximum uncertainty in the calculations. The analysis was performed in Crystal Ball® Pro software after running 10,000 Monte Carlo iterations for each model. The dosing unit of toxin concentration (Model 1) and model parameter uncertainties (q1 and q2) contributed approximately 63% to 96% of risk uncertainties in the calculations, as compared to environmental sampling uncertainties. However, in Model 2, the variability in the measurements of indoor spore concentration was found to have approximately 97% influence in risk uncertainties. This is an interesting finding as it further emphasizes on the fact that environmental sampling of S. *chartarum* spores is involved with lots of variability which significantly affects our risk estimates. However, with toxins as exposure-indicators the intrinsic model variability dominates.

The sensitivity analysis results provide an insight on the current strength and limitations of S. *chartarum* QRA. The use of animal dose-response studies for human projections should be reviewed with caution. Various studies using the same species of rats and mice have shown a broad range of variation in the dose-response dataset due to differing *S. chartarum* strains and infant subject respiratory responses to mycotoxins. We believe that studies with more dose groups and sufficient animals per dose group are needed for precise determination of dose-response curves. Furthermore, detailed mechanism-based modeling could be applied on the physiological, biochemical, and bronchoalveolar lavage fluid (BALF) response information to offer a better understanding of dose dependence in animals.

Summary of case reports and mortality rates for AIPH

In 2001, the CDC published the case definition of AIPH and began investigating potential reported individual and cluster cases of AIPH [42]. The first reported case since 2001 occurred in Boston, Massachusetts, between December 2002 and June 2003. Four cases of AIPH among full-term infants were admitted to intensive care units and discharged in good health after an approximate week-long stay. It reported that S. *chartarum* spores were present in two of the cases homes with quantifications of one and seven spores, but no toxin levels were provided [43].

In the Cleveland study, the reported total number of hospitalizations was 30 with a mortality rate of 34%. The total number of exposed infants was not provided and could not be used to further validate model predictions [14]. Since the Cleveland study, there have been individual reports of AIPH in infants who were living in mold contaminated environment, but not every

environment contained S. *chartarum* [44]. The most recent Morbidity and Mortality Weekly Report (MMWR) on AIPH was published in March 2004, with the CDC retrospectively reviewing cases of pulmonary hemorrhage to determine the public health impact of AIPH among infants. These review cases are also used to generate hypotheses regarding the importance of potential risk factors that may contribute to AIPH in infants. It is a challenge to estimate the mortality rate in exposed groups from review cases that occurred before 2001, due to the availability of hospital records only at the national level and lack of clinical information to confirm AIPH based on the definition [42,43]. Due to these challenges, risk estimates would benefit from a thorough epidemiological study on the effects of *S. chartarum* in infants for validity of proposed models.

Data Gaps

Large gaps remain in the knowledge base needed to conduct quantitative risk assessments for inhaled mycotoxins associated with *S. chartarum* spores. The result of this research indicates that a health hazard after inhalation of SC- toxins exists in human infants. To what extent are these risk estimates relevant, requires more thorough investigation of indoor environmental fungal aura and reported outbreak/case studies. After intense literature search few case reports were found reporting AIPH under damp indoor environment but they failed to provide quantitative information on human exposure group or mycotoxin exposure levels in the indoor environment. Furthermore, there are many uncertainties associated when animal data is extrapolated to humans. Some of the key factors are briefly summarized below:

Acute versus chronic effect: Studies have reported toxic effects with repeated exposure to toxins, suggesting that the peak effect may be cumulative. A chronic exposure study may provide a more well-rounded representation of human risk [24,20].

Toxin/spore quantification: Concern about inefficient detection methods of toxins and spores has been discussed. In conjunction with new specificity and sensitivity testing methods such as the qPCR, additional research is needed to ensure less cross-connectivity in assay results [45]. Viable spore quantification is dependent on the selection of appropriate culture media, but the effect of different media across studies could not be evaluated while developing the dose-response models within this study.

Other fungal component of spores: The role of other fungal components in response is not defined/analyzed while using the models based on pure toxin instillation (Model 1).

Characterization of fungal spores: Viability and trichothecene content of fungal spores are the critical factors in determining the exposure level in animals and humans. Intra-strain variation in toxin content of spores makes it complicated to precisely quantify the exposure levels in terms of spore concentrations. Moreover, the viable spores are reported to germinate in lungs and caused greater mortality than non-viable spores in rats at comparable doses [46]. Thus, indicating that predictions based on animal studies where only non-viable spores were given may have under-reported the risk.

Model validation: For better comparison between model estimates and real world scenarios, only mortality rates were found in case reports. Due to lack of data on ambient level of toxins/spores present at time of exposure, it was not possible to verify if the produced risk estimates are relevant.

Conclusion

Several studies indicate that the inhalation of high indoor mold spore concentrations results in one or more severe health hazards [47]. However, the extent to which these molds are responsible for illness or death of inhabitants is not entirely clear. The causal relationship between mold exposure and health risk is confounded by the presence of other environmental substances, pre-existing health conditions, and variation in respiratory response in subjects. Despite the difficulty in establishing extent, *Stachybotrys chartarum* has become a public health concern due to its ability to produce mycotoxins. It is elucidated by numerous animal studies that acute exposure to S. *chartarum* toxins results in animal infant mortality due to the susceptibility of their immature and developing lungs. We assumed the use of these animal dose-response models in predicting mortality risk in human infants due to the morphologically similar responses between mice and monkeys at comparable doses of S. *chartarum* toxin.

There is a significant lack of meaningful data relative to the human health effects of airborne exposure to mycotoxins. Although comprehensive data are available from animal inhalation studies and cluster reports are available on AIPH in infants, it is complicated to

demonstrate a definitive link between exposure to mycotoxins and pulmonary response. For many of the biological responses elicited by *S. chartarum*, Schulz, Senkpiel et al. acknowledge that animal-to-human correlation would be strengthened by additional mechanistic data, such as the relevance of specific biological precursors to toxic responses, inflammatory responses, and serum antibodies levels risks [47]. Such additional information would greatly expand data sets for dose-response, as many of the data sets in the literature was based on biomarkers and thus could not be used in a standard dose-response model.

The results of our analysis exhibited that there is a wide range of infant mortality risk for acute 24h exposure to toxins, with lowest value as 1.0×10^{-11} to maximum average as 3.0×10^{-6}. The risk projections presented in this work are based on developed dose-response models using both toxins and spores as exposure units, providing a more robust QRA framework than previous approaches of using animal derived NOAEL/LOAEL values to inform on health hazard. However, contrasting results between spores and toxins risk projections exist in our analysis. One projection (toxin) resulted in exceptionally high risk estimates while the other (spore) indicated that current exposure levels are safe. It is important to remember that major data gaps existed in the QRA. Furthermore, the prediction of benchmark dose in this study is presented merely as a quantitative framework for policy makers to establish guidelines for regulatory action under adverse conditions.

In summary, we conclude that application of quantitative risk assessment framework for characterizing health hazard from indoor mold and toxins can potentially provide a tool to evaluate the extent of mold threat and lay a foundation for regulatory supervision of infrastructures. Advanced detection and sampling methods are needed in order to enhance our confidence in such approaches. The results indicated that while toxin exposure is inconclusive for AIPH in infants, substantial health risk after inhalation of SC-toxins/spores exists in human infants and a conclusive epidemiology study is needed to validate these predicted risks. Given the controversies surrounding the exposure and risk characterization of SC molds, these estimates should be interpreted with caution. Additionally, we suggest that the hypotheses of insignificant health risk to infants under the exposure conditions commonly encountered in a moldy indoor environment is totally based on spore quantification and may drastically under-report the disease burden associated with *Stachybotrys* fungi.

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