Journal of Toxicology and Environmental Health, Part A, 72: 585–598, 2009
Copyright © Taylor & Francis Group, LLC
ISSN: 1528-7394 print / 1087-2620 online
DOI: 10.1080/15287390802706389

Taylor & Francis
Taylor & Francis Group

The Concentration of No Toxicologic Concern (CoNTC) and Airborne Mycotoxins

Bryan D. Hardin[1], Coreen A. Robbins[1], Payam Fallah[2], and Bruce J. Kelman[1]
[1]*Veritox®, Inc., Redmond, Washington, and* [2]*Indoor Environmental Hygiene Laboratory (IDEHL®), Redmond, Washington, USA*

The threshold of toxicologic concern (TTC) concept was developed as a method to identify a chemical intake level that is predicted to be without adverse human health effects assuming daily intake over the course of a 70-yr life span. The TTC values are based on known structure–activity relationships and do not require chemical-specific toxicity data. This allows safety assessment (or prioritization for testing) of chemicals with known molecular structure but little or no toxicity data. Recently, the TTC concept was extended to inhaled substances by converting a TTC expressed in micrograms per person per day to an airborne concentration (ng/m^3), making allowance for intake by routes in addition to inhalation and implicitly assuming 100% bioavailability of inhaled toxicants. The resulting concentration of no toxicologic concern (CoNTC), 30 ng/m^3, represents a generic airborne concentration that is expected to pose no hazard to humans exposed continuously throughout a 70-yr lifetime. Published data on the levels of mycotoxins in agricultural dusts or in fungal spores, along with measured levels of airborne mycotoxins, spores, or dust in various environments, were used to identify conditions under which mycotoxin exposures might reach the CoNTC. Data demonstrate that airborne concentrations of dusts and mold spores sometimes encountered in agricultural environments have the potential to produce mycotoxin concentrations greater than the CoNTC. On the other hand, these data suggest that common exposures to mycotoxins from airborne molds in daily life, including in the built indoor environment, are below the concentration of no toxicologic concern.

Modern analytical chemistry permits the detection of vanishingly small amounts of natural and synthetic substances, whether in air, water, food, cosmetics, or pharmaceuticals. For most of these substances, the available toxicology data are inadequate to support a conventional safety evaluation or risk characterization. It was recognized for at least 40 yr (Frawley, 1967; Munro, 1990) that a method was needed to establish thresholds for regulatory concern to determine that exposures are low enough to be confident that they pose no toxicologic concern or, conversely, to determine that exposures are such that a safety evaluation requires chemical-specific toxicologic data.

The threshold of toxicologic concern (TTC) concept developed as a means to make those determinations. The TTCs identify, with a wide margin of safety, a level of intake that is predicted to be without adverse human health effects assuming daily intake at or below the TTC over the course of a 70-yr life span. The TTCs are based on known structure–activity relationships and their application does not require chemical-specific toxicity data. This allows safety assessment (or prioritization for testing) of chemicals with known molecular structure but little or no toxicity data.

A decision tree approach to estimating toxic hazard was proposed in 1978 for chemicals with known molecular structure (Cramer et al., 1978). Consisting of "yes" or "no" responses to a series of 33 questions about molecular structure, the decision tree sorted substances to one of three categories, I, II, or III. Class I substances were described as having molecular structures and toxicity data suggestive of low oral toxicity, while Class III substances were described as lacking any strong presumption of safety or with molecular indicators of possible significant toxicity. Class II was described as less clearly innocuous than Class I, but also lacking the molecular indicators of toxicity that characterized Class III. Applying the decision tree requires a sophisticated knowledge of chemistry. However, the Computational Toxicology Group of the European Chemicals Bureau developed and makes available (http://ecb.jrc.it/qsar/) a user-friendly open-source application, Toxtree, that places chemicals into the appropriate Cramer classifications (Patlewicz et al., 2008).

Based on an analysis of the potencies of 477 carcinogens, in 1995 the U.S. Food and Drug Administration (FDA) modified

Received 26 March 2008; accepted 5 December 2008.

All of the authors have published previously on exposures to fungi and hazards to human health associated with those exposures. All of the authors have provided consultation services regarding indoor air quality and have provided expert testimony in litigation.

Neither the authors nor their employers, Veritox,® Inc. and IDEHL,® received financial assistance or compensation of any kind for preparation of this publication.

Address correspondence to Bryan D. Hardin, PhD, FATS, Veritox,® Inc., 18372 Redmond-Fall City Road, Redmond, WA 98052, USA. E-mail: BHardin@Veritox.com

the food additive regulations to establish a "threshold of regulatory concern" for substances that migrate to food from packaging (FDA, 1995). These regulations exempt substances from regulation as food additives when dietary concentrations are less than 0.5 ppb, "corresponding to a daily dietary intake of 1.5 μg/person/day or less (based on a diet of 1,500 g of solid food and 1,500 g of liquid food per day)" (21 CFR 170.39(a)(2)(ii)). In its rulemaking decision, the FDA concluded that even if "an exempted substance present in the diet at 0.5 ppb were later found to be a carcinogen, the upper-bound lifetime risk resulting from the use of the substance is likely to be below one in a million," a risk level that was considered as posing "only negligible safety concerns" (FDA, 1995).

The TTC concept was proposed (Munro et al., 1996a, 1996b) as an extension of the FDA regulatory analysis and the previously published (Cramer et al., 1978) decision tree categorization by molecular structure. Based on an analysis of over 2900 noncancer no-observed-effect levels (NOELs) in toxicology studies, the fifth percentile NOEL was identified for Cramer classes I, II, and III: 3000, 910, and 150 μg/kg/d, respectively. These NOEL were reduced by a 100-fold safety factor and became the basis for suggested thresholds of concern for daily human intakes (assuming a human body weight of 60 kg): Class I, 1800 μg/d; Class II, 540 μg/d; and Class III, 90 μg/d (Table 1). These TTC were suggested to apply to Cramer class I, II, and III chemicals having no presumption of genotoxic carcinogenicity. The FDA-adopted threshold of 1.5 μg/d was considered more appropriate for chemicals with that presumption. Since this initial proposal, the TTC concept was refined, its scientific support strengthened, and it was used internationally, for example, by the Joint FAO/WHO Expert Committee on Food Additives (Kroes et al., 2000, 2004, 2005; Kroes & Kozianowski, 2002; Munro et al., 1999; Renwick, 2005).

An international Expert Group (Kroes et al., 2000; Kroes & Kozianowski, 2002) was convened to evaluate whether TTCs based on Cramer classes would provide adequate protection for potentially sensitive noncancer endpoints, i.e., neurotoxicity, neurodevelopmental toxicity, developmental toxicity, immunotoxicity, and endocrine effects. The Cramer class III TTC of 90 μg/d was found protective for all except neurotoxicity. A TTC of 18 μg/d was proposed for neurotoxicity, driven by the toxicity of organophosphate (OP) esters. The Expert Group affirmed that chemicals in the diet pose no appreciable risk if consumed at levels below 1.5 μg/d, and suggested that higher TTCs would be appropriate for compounds that lack structural alerts for genotoxicity and carcinogenicity.

Subsequently, the Expert Group developed a decision tree to guide implementation of TTCs in food safety evaluation (Kroes et al., 2004), incorporating the previously recommended TTCs of 1800, 540, and 90 μg/d for chemicals in Cramer categories I, II, and III, respectively. This decision tree

TABLE 1
Thresholds of Toxicologic Concern (TTC) for Human Exposure

Chemical class/ toxicologic effect	TTC		Corresponding dietary concentration[c](ppb)	Reference
	μg/kg per day[a]	(μg/person per d)[b]		
Cramer class I	30	1800	600	(Kroes et al., 2000, 2004; Kroes & Kozianowski, 2002; Munro et al., 1996a)
Cramer class II	9	540	180	(Kroes et al., 2000, 2004; Kroes & Kozianowski, 2002; Munro et al., 1996a)
Cramer class III	1.5	90	30	(Kroes et al., 2000, 2004; Kroes & Kozianowski, 2002; Munro et al., 1996a)
Neurotoxicity/ organophosphates	0.3	18	6	(Kroes et al., 2000, 2004)
Indirect food additives	0.025	1.5	0.5	(FDA, 1995)
Genotoxicity structural alerts	0.0025	0.15	0.05	(Kroes et al., 2004)

[a]5th percentile class NOEL reduced by 100-fold safety factor.
[b]Assumes 60 kg body weight.
[c]Corresponding to a daily dietary intake of 1500 g of solid food and 1500 g of liquid food as assumed by the US FDA in 21 CFR 170.39(a)(2)(ii).

also incorporated two supplemental TTCs: one for OP esters (18 μg/d) and one for chemicals with structural alerts for genotoxicity (0.15 μg/d) (Table 1). This last TTC was based on an analysis of 730 high-potency carcinogens with linear extrapolation to the upper bound estimate of the 1 in 1 million lifetime cancer risk. The Expert Group also concluded that risk assessments should require compound-specific toxicity data anytime a chemical would exceed its TTC and for *N*-nitroso-, azoxy-, and aflatoxin-like compounds. Guidance levels for aflatoxins and various other mycotoxins in foods are established in almost 100 nations (FAO, 2004), including the developed nations shown in Table 2.

It was recognized that, with appropriate adjustments for differing routes of intake, the TTC concept can be applied to more than dietary exposures (Kroes et al., 2005). Proposals recently were made to apply the TTC concept for safety evaluations of contaminants in personal and household care products (Blackburn et al., 2005), in pharmaceuticals (Ball et al., 2007; Dolan et al., 2005; Humfrey, 2007; Müller et al., 2006), in cosmetic ingredients (Kroes et al., 2007), in soil (Wilson et al., 2000), for dermal sensitization (Safford, 2008), and in ambient air (Drew & Frangos, 2007). A more detailed discussion of the development and implementation of the TTC concept and its more recent extension to new applications can be found in Munro et al. (2008).

Extending the TTC concept to inhaled chemical substances requires conversion of a TTC expressed in micrograms per person per day to an airborne concentration ($\mu g/m^3$) that would deliver an inhaled dose equivalent to the TTC oral dose. The concentration of no toxicological concern (CoNTC) was derived (Drew & Frangos, 2007) using 1.5 μg/d, the regulatory threshold (21 CFR 170.39) for indirect food additives, as the point of departure. For a 60-kg adult, this TTC is equivalent to 0.025 μg/kg body weight/d, but because there might be sources of intake other than inhalation, the CoNTC was developed to deliver a daily dose of only 0.01 μg/kg/d. Age-specific respiratory rates and body weights were applied in a mathematical model to derive a lifetime average CoNTC of 30 ng/m^3. This generic airborne concentration was proposed as a screening level for industrial emissions to ambient air, below which there would be no hazard to humans exposed continuously throughout a 70-yr lifetime.

Mycotoxins are secondary metabolic products of filamentous fungi, or molds, many of which routinely contaminate human and animal food supplies. Mycotoxins in food are recognized as the cause of acute and chronic adverse health effects in humans and animals. Mycotoxins may be present in agricultural environments where airborne concentrations of dusts and mold spores may be high. The aim of this study was to investigate the possibility that airborne concentrations of mycotoxins in these environments may exceed the CoNTC.

METHODS

Over 1200 published papers on molds and mycotoxins were screened to identify reported airborne concentrations of mold spores or dusts in agricultural and other occupational environments. These published exposure data provide an overview of

TABLE 2
Representative Guidance Levels (ppb) in Developed Nations for Mycotoxins in Food (FAO, 2004)

Mycotoxin	Canada	European Union	Japan	U. S.
Aflatoxins B_1 B_2 G_1 G_2	15 Nuts and nut products	4 Groundnuts, dried fruit, cereals, and processed products made from them intended for direct human consumption	10 (AFB_1) All foods	20 All foods except milk
Aflatoxin M_1		0.05 Milk		0.5 Milk
Deoxynivalenol (DON)	1200 (adult foods) 600 (infant foods) Wheat flour	500 Cereal products as consumed or at retail stage	1100 Wheat and wheat products	1000 Finished wheat products for consumption by humans
Fumonisins B_1 B_2 B_3				2000–4000 corn products (varies according to type of corn product)
Ochratoxin A		3 Cereal grain products intended for human consumption	50 Apple juice	
Patulin	10 Apple juice, solid apple products, baby foods			50 Apple juice

the range of airborne spore and dust concentrations that can be encountered in these environments. In addition, reported concentrations of specific mycotoxins were identified as measured directly in the air, in mold spores, or in agricultural or other dusts that might become aerosolized. These published data on exposure and on the mycotoxin content of dusts and spores were used to estimate conditions under which the airborne mycotoxins might reach or exceed the CoNTC.

RESULTS

Measured Exposures

Humans experience inhalation exposure to molds and potentially to mycotoxins in many environments but most significantly in agricultural settings such as farming, dairy operations, animal confinement, grain handling, and waste composting. In these environments concentrations of airborne particulate matter, including mold spores, may be high. For example, counts of total fungal spores in these occupational environments may reach 10^9 to 10^{10} spores/m^3 (Halstensen et al., 2007; Malmberg et al., 1993; Melbostad & Eduard, 2001; Palmgren et al., 1986) (Table 3). Total dust concentrations approach or exceed 100 mg/m^3, and highs over 600 mg/m^3 were encountered in some agricultural activities (Halstensen et al., 2007; May et al., 1986; Morey, 1990; Sorenson et al., 1984; Todd & Buchan, 2002).

Mycotoxins may be present in agricultural dusts at relatively high levels (Table 4), representing mycotoxins present in various fungal elements present in the dust, e.g., spores, mycelia and their fragments, and mycotoxins excreted into or onto the aerosolized growth substrate. Settled dust from cowsheds contained ochratoxin A at up to 70 μg/kg dust (Skaug et al., 2000). If those dusts were aerosolized the airborne ochratoxin concentration would be 7 ng/m^3 at 100 mg dust/m^3, ranging up to 35 ng/m^3 at 500 mg dust/m^3. Settled grain dust was analyzed for 8 mycotoxins and 3 were detected, with the maximum being HT-2 toxin at 2400 μg/kg dust (Nordby et al., 2004). If that dust were aerosolized the HT-2 concentration would be 240 ng/m^3 at 100 mg dust/m^3, reaching 1200 ng/m^3 at 500 mg dust/m^3. Dust collected from walls and equipment in a swine nursery building contained up to 5 ng aflatoxin B_1/mg dust (Selim et al., 1998). The airborne aflatoxin concentration would be 500 ng/m^3 at 100 mg dust/m^3, or 2500 ng/m^3 at 500 mg dust/m^3.

Although data are limited, attempts have been made to measure airborne mycotoxin concentrations directly (Table 5). In occupational environments, reliable chromatographic techniques were used to determine airborne concentrations of 7.6 to 421 ng aflatoxins/m^3 (Burg et al., 1981, 1982; Selim et al., 1998; Sorenson et al., 1984) and 8.15 ng ochratoxin A/m^3 (Iavicoli et al., 2002). Nonspecific, unvalidated methods were used with indoor air samples, but reported airborne concentrations were 0.12 to 1.37 ng "macrocyclic trichothecenes"/m^3 (Brasel et al., 2005), 18 ng "T-2 toxin equivalents"/m^3, and 34 ng "satratoxin G equivalents"/m^3 (Yike et al., 1999).

Mycotoxin Content of Spores

Concentrations of mycotoxins in air can be estimated indirectly based on published mycotoxin concentrations in spores. That approach was used for a small number of mycotoxins for which both mycotoxin per spore and dose-response data were available (Kelman et al., 2004). These authors estimated doses humans might inhale in 24 h and then compared those doses to published dose responses. Because comparison to the CoNTC does not require compound-specific dose-response information, a larger number of mycotoxins can be considered here (Table 6), where per-spore concentration of mycotoxins is seen to range over 5 orders of magnitude (10^{-3} to 10^{-8} ng/spore).

The mycotoxin per spore concentrations shown in Table 6 represent the highest concentration reported if more than one fungal species, strain, or isolate was evaluated, or if mycotoxin production was evaluated on more than one defined lab medium. Some of the mycotoxin concentrations shown in Table 6 were determined with unvalidated or nonspecific methods. It is important to note as well that all of these mycotoxin per spore concentrations were derived with laboratory cultures grown on defined medium. It is not known whether these concentrations are representative of maximal concentrations that can be achieved outside the lab with growth on natural substrates, where it is known that mycotoxins are not produced at all times or under all conditions (Chapman, 2003; Rao et al., 1996, 2001; Tuomi et al., 2000).

Airborne spore numbers are reported to be significantly exceeded by the numbers of fungal fragments that are smaller than spores (Cho et al., 2005; Górny et al., 2001, 2002), and it is possible for those fragments to contribute to the airborne concentration of mycotoxins. Reports of small fragments were based on an aggressive sampling method in which culture dishes were placed on an operating vortex mixer, enclosed in a shroud with air jets directed downward onto the surface, and particles were collected. Smaller particles were reported to outnumber spores by 320:1 (Górny et al., 2001) to 500:1 (Cho et al., 2005), and particle sizes were reported to range down to 0.03 μm aerodynamic diameter (Cho et al., 2005). Neither the numbers nor the size distribution of particles generated in this way was shown to reflect particles released under natural conditions. Based on the formula for the volume of a sphere ($4/3 \cdot \pi \cdot r^3$) and the published volume (2.8×10^{-10} cm^3) of a *Stachybotrys chartarum* spore (Burge, 1996), it can be seen that for spheres with a diameter of 0.3 μm (1.4×10^{-14} cm^3 volume), 20,000 are required to equal the volume of a single *S. chartarum* spore. If these particles are not spheres, then an even larger number would be required to achieve the same volume. With a numerical excess of less than 500:1, including particles smaller than 0.3 μm, it is unlikely that these small fragments add meaningfully to the airborne mycotoxin concentration attributable to spores.

TABLE 3
Spore Counts in Occupational Environments

Published source	Environment sampled	Activity	Airborne concentration
(Baruah, 1961)	Cowshed—peak generation when distributing hay		Spores/m^3
		Counts during	Average Total (Pen / Asp)
		Peak Generation	16,497,000 (12,390,000)
		Off-Peak Generation	95,200 (32,600)
(Darke et al., 1976)	Grain harvesting	Breathing zone samples for combine operator	500,000–34,000,000
(Duchaine et al., 2000)	Sawmills	Debarking operation	CFU/m^3 on Czapek agar
			Range 600–2,100,000
(Durand et al., 2002)	Composting facilities		Spores/m^3
		Municipal biosolids	93,325,400
		Yard waste	1,230,300
		Cow manure	1,862,100
(Eduard et al., 2001)	PBZ samples of farm families	Harvesting, tending animals, and handling manure	Spores/m^3 8-h TWA
			Arithmetic Mean 1,200,000
			Geometric Mean 99,000
(Halstensen et al., 2007)	Various farm activities, breathing zone samples	During grain harvesting	Spores/m^3
			Highest sample 5,200,000,000
			Arithmetic mean 62,000,000
			Geometric mean 4,000,000
(Heldal et al., 2003)	Household waste collectors	Driving trucks; emptying waste containers	Spores/m^3
			Median 170,000
			Range 0–2,000,000
(Heldal & Eduard, 2004)	Household waste collectors	Driving trucks; emptying waste containers	Spores/m^3
			Arithmetic mean 320,000
			Range 0–2,300,000
(Herr et al., 2003)	Residential neighborhood near composting site creating bioaerosol pollution of outdoor air	Ambient air	CFU/m^3 on DG18
		Upwind 500 m	1900–3,600
		Downwind 200 m	7700–130,000
		300 m	4300–17,000
		550 m	2300–4100
(Lappalainen et al., 1996)	Area samples on 8 Finnish farms		Spores/m^3
		Drying grain	Range 1,000,000–3,700,000
		Milling grain	Range 280,000–23,000,000
		Feeding cattle	Range 870,000–9,800,000
(Lee et al., 2006)	Breathing zone sampling on farms in animal confinement facilities and during harvest	Summertime sampling:	Mean spores/m^3
		Swine	14,200
		Poultry	18,000
		Dairy	36,000
		Fall harvest	
		Corn	6,100,000
		Soybean	830,000
(Malmberg et al., 1993)	PBZ samples collected during activities believed associated with prior AA or ODTS	Farms with history of:	Spores/m^3
		No respiratory disease	100,000,000
		Allergic alveolitis (AA)	2,600,000,000
		ODTS	13,500,000,000
(Melbostad & Eduard, 2001)	Activities frequently associated with symptomatic complaints		Geometric mean Spores/m^3
		Threshing	900,000
		Handling grain	2,000,000
		Handling hay	1,900,000
		Tending swine	3,100,000
		Tending poultry	300,000
(Morey, 1990)	Inside silo	Unloading cap silage	Maximum CFU/m^3 160,000,000
(Palmgren et al., 1986)	Various farming activities	Peak levels of total micro-organisms	Range: Counts/m^3 920,000,000–24,000,000,000

Note. AA, allergic alveolitis; CFU, colony-forming units; DG18, dichloran 18% glycerol agar; ODTS, organic dust toxic syndrome; PBZ, personal breathing zone; Pen/Asp, *Penicillium*/*Aspergillus*; TWA, time-weighted average.

TABLE 4
Mycotoxins in Dusts

Published source of mycotoxin data	Mycotoxin	ng mycotoxin per mg dust	ng mycotoxin/m^3 at OSHA PEL for Total Dust[a]	Comments
(Bloom et al., 2007)	Trichodermol	0.003	0.05	8 dust samples from 4 water-damaged homes in Sweden analyzed for verrucarol and trichodermol by GC-MS, and for sterigmatocystin, satratoxin G, and satratoxin H by HPLC-MSMS. No detectable mycotoxin in 5/8 samples; 2/8 positive for verrucarol, 2/8 for trichodermol, 1/8 for sterigmatocystin
	Sterigmatocystin	0.017	0.26	
	Verrucarol	0.043	0.06	
(Skaug et al., 2000)	Ochratoxin A	0.07	1.0	Dust samples collected from 14 cowsheds and analyzed for ochratoxin A by HPLC. OTA detected in 6 of 14 samples at 0.2 to 70 μg OTA/kg dust
(Nordby et al., 2004)	Deoxynivalenol	0.34	5.1	Maximum measured mycotoxin content in 104 settled dust samples on Finnish farms during grain production. Another 5 trichothecenes were analyzed for and maxima found were 67 μg/kg (0.067 ng/mg) or less as determined by GC-MS
	T-2 toxin	1.2	18.0	
	HT-2	2.4	36.0	
(Selim et al., 1998)	Aflatoxin B_1	5.1	76.5	9 samples of settled dust collected from walls and equipment in swine nursery building; analyzed by HPLC; AFB_1 concentrations ranged from 23 to 5,101 ng/g dust.

Note. AFB_1 - aflatoxin B_1; GC-MS - gas chromatography–mass spectroscopy; HPLC-MSMS - High performance liquid chromatography-tandem mass spectroscopy; OTA- ochratoxin A.

[a]29 CFR 1910.1000 Table Z-1 Permissible Exposure Limit (PEL) for particulates not otherwise regulated, total dust 15 mg/m^3.

Comparisons to the CoNTC

As shown in Table 4, even if dust exposures were limited to OSHA's occupational limit for total dust, some of the mycotoxin-containing dusts would generate airborne concentrations of some mycotoxins (aflatoxin B_1 and HT-2 toxin) in excess of the CoNTC. Under the most extreme conditions of dustiness in agricultural operations, most of these dusts could give rise to airborne mycotoxin concentrations above the CoNTC and worker exposures above the CoNTC were documented in grain handling operations (Table 5).

None of the publications that reported the concentration of mycotoxin on a per-spore basis (Table 6) included actual exposure data. However, Table 6 includes for each of the entries the calculated number of those spores in air that would be required to reach the CoNTC. With mycotoxin levels per spore ranging over 5 orders of magnitude, 10^3 to 10^9 of these spores per

TABLE 5
Measured Concentrations of Mycotoxins in Air

Published source	Mycotoxin	ng/m^3	Environment sampled
(Brasel et al., 2005)	Macrocyclic trichothecenes	0.12	Building with no known mold or water intrusion. Mycotoxin determined by QuantiTox ELISA kit.
(Brasel et al., 2005)	Macrocyclic trichothecenes	1.37	Aggressive sample in home with visible mold and water intrusion. Mycotoxin determined by QuantiTox ELISA kit.
(Sorenson et al., 1984)	Aflatoxin B_1	7.60	Samples from a peanut processing plant; dust ranged from 10.5 to 65.1 mg/m^3. AFB_1 quantified by column chromatography followed by 2-D TLC
(Iavicoli et al., 2002)	Ochratoxin A	8.15	Warehouses processing coffee, black pepper, nutmeg, cocoa beans. Highest of 18 area and PBZ samples. OTA quantified by HPLC
(Yike et al., 1999)	T-2 toxin "equivalents"	18	Sample from the bedroom of a Cleveland home. "T-2 toxin equivalents" determined by luciferase translation inhibition assay
(Yike et al., 1999)	Satratoxin G "equivalents"	34	Sample from the bedroom of a Cleveland home. "SG equivalents" determined by luciferase translation inhibition assay
(Burg et al., 1982)	Aflatoxins B_1+B_2	55	Sample from a grain elevator during unloading of 1-yr-old aflatoxin-contaminated corn. Aflatoxins quantified by column chromatography followed by 1- or 2-D TLC
(Burg et al., 1981)	Aflatoxins B_1+B_2	107	Sample from inside a storage bin during handling of aflatoxin-contaminated corn. Aflatoxins quantified by column chromatography followed by 1-D TLC
(Selim et al., 1998)	Aflatoxin B_1	421	Sample from an enclosed swine facility, associated with a dust concentration of 2.1 mg/m^3. AFB_1 quantified by column chromatography followed by TLC or HPLC

Note. AFB_1, aflatoxin B_1; ELISA, enzyme-linked immunosorbent assay; HPLC, high-performance liquid chromatograpy; TLC, thin-layer chromatograpy; OTA, ocharatoxin A; PBZ, personal breathing zone; 1-D, one-dimensional; 2-D, two-dimensional.

cubic meter of air would be required to produce an airborne mycotoxin concentration equal to the CoNTC of 30 ng/m^3.

DISCUSSION

Over 400 mycotoxins are known and it is reasonable to expect many more mycotoxins remain to be identified. Only limited toxicologic data are available for a small number of the known mycotoxins, and most of those data derive from human or animal dietary exposures. Adequate mycotoxin-specific toxicologic data will not soon be available for even the most important of the known mycotoxins. Some method is needed to recognize exposure conditions in which inhalation of mycotoxins might represent a potential hazard calling for exposure reduction or compound-specific safety assessment. The TTC concept and the CoNTC as proposed by Drew and Frangos (2007) offer an approach to making that judgment.

TABLE 6
Minimal Spore Concentrations to Reach the CoNTC (30 ng/m^3) of Mycotoxins

Published source of mycotoxin data	Mold species	Mycotoxin	Maximum ng mycotoxin per spore	Minimum Spores/m^3 to reach CoNTC[a]	Comments
(Palmgren & Lee, 1986)	*Aspergillus parasiticus* mutant Nor-1	Norsolorinic acid	2.3×10^{-8}	1,304,347,826	SRRC-162 cultured on rice. Mycelial matrix plus substrate and spores contained 735 µg/g as determined by TLC
(Palmgren & Lee, 1986)	*Penicillum oxalicum*	Secalonic acid D	2.5×10^{-8}	1,200,000,000	SRRC-2007 cultured on rice. Mycelial matrix plus substrate and spores contained 488 µg/g as determined by HLPC
(Palmgren & Lee, 1986)	*Aspergillus niger*	Aurasperone C	1.1×10^{-7}	263,157,895	SRRC-2005 cultured on rice. Mycelial matrix plus substrate and spores contained 110 µg/g as determined by TLC
(Land et al., 1994)	*Aspergillus fumigatus*	Fumitremorgen B	9.0×10^{-7}	33,333,333	Isolate K from Swedish sawmills, cultured on YES medium. 0.9 µg/10^9 condia, determined by HPLC
(Palmgren & Lee, 1986)	*Aspergillus parasiticus*	Aflatoxin B_1	9.8×10^{-7}	30,737,705	SRRC-2004 cultured on rice. Mycelial matrix plus substrate and spores contained 242 µg/g as determined by TLC
(Sorenson et al., 1987)	*Stachybotrys chartarum*	Trichoverrols A+B	1.5×10^{-6}	20,238,095	*S. atra* Debrecen 1132 strain grown in rice culture. Aerosols generated, collected, and ng mycotoxin/mg aerosol determined by HPLC. Concentration per spore calculated assuming *S. atra* spores weigh 0.28 ng/spore (Burge, 1996) and that all trichoverrols (4.5 ng/mg) were in spores (85% of aerosol mass)
(Land et al., 1994)	*Aspergillus fumigatus*	Verruculogen	2.2×10^{-6}	13,636,364	Isolate B from Swedish sawmills, cultured on YES medium. 2.2 µg/10^9 condia, determined by HPLC
(Sorenson et al., 1987)	*Stachybotrys atra*	Satratoxin G	2.3×10^{-6}	13,198,758	*S. atra* Debrecen 1132 strain grown in rice culture. Aerosols generated, collected, and ng mycotoxin/mg aerosol determined by HPLC. Concentration per spore calculated assuming *S. atra* spores weigh 0.28 ng/spore (Burge 1996) and that all satratoxin G (6.9 ng/mg) was in spores (85% of aerosol mass)
(Sorenson et al., 1987)	*Stachybotrys atra*	Satratoxin H	4.2×10^{-6}	7,170,979	*S. atra* Debrecen 1132 strain grown in rice culture. Aerosols generated, collected, and ng mycotoxin/mg aerosol determined by HPLC. Concentration per spore calculated assuming *S. atra* spores weigh 0.28 ng/spore (Burge, 1996) and that all satratoxin H (12.7 ng/mg) was in spores (85% of aerosol mass)

(*Continued*)

TABLE 6
(*Continued*)

Published source of mycotoxin data	Mold species	Mycotoxin	Maximum ng mycotoxin per spore	Minimum Spores/m^3 to reach CoNTC[a]	Comments
(Sorenson et al., 1987)	*Stachybotrys atra*	Satratoxins G+H Trichoverrols A+B	7.5×10^{-6}	3,976,918	*S. atra* Debrecen 1132 strain grown in rice culture. Aerosols generated, collected, and ng mycotoxin/mg aerosol determined by HPLC. Concentration per spore calculated assuming *S. atra* spores weigh 0.28 ng/spore (Burge, 1996) and that all mycotoxins (22.9 ng/mg) were in spores (85% of aerosol mass)
(Palmgren & Lee, 1986)	*Aspergillus fumigatus*	Fumigaclavine C	9.9×10^{-6}	3,033,367	SRRC-2006 cultured on rice. Mycelial matrix plus substrate and spores contained 39 μg/g as determined by TLC
(Land et al., 1994)	*Aspergillus fumigatus*	Fumitremorgen C	1.2×10^{-5}	2,586,207	Isolate B from Swedish sawmills, cultured on YES medium. 11.6 μg/10^9 condia, determined by HPLC
(Land et al., 1994)	*Aspergillus fumigatus*	Fumitremorgens B and C and verruculogen	1.4×10^{-5}	2,083,333	Isolate B from Swedish sawmills, cultured on YES medium. 14.4 μg/10^9 condia, determined by HPLC
(Ren et al., 1999)	*Aspergillus flavus*	Aflatoxin B_2	3.6×10^{-5}	833,333	NRRL-3251 cultured on glucose yeast extract agar—highest production of 8 strains evaluated. No significant mycotoxin production by any strain grown on building materials. Aflatoxin determined by TLC and HPLC
(Skaug et al., 2000)	*Aspergillus ochraceus*	Ochratoxin A	6.0×10^{-5}	500,000	IBC 5075 cultured on Sabouraud agar. OTA determined by HPLC
(Land et al., 1993)	*Aspergillus fumigatus*	Fumitremorgens + verruculogen	8.0×10^{-5}	375,000	73 strains from 21 Swedish sawmills, mycotoxin production on YES medium (no mycotoxins recovered when grown on wood); 7 produced tremorgens, range 0.6–8.0 μg/10^8 conidia, determined by 2-D TLC
(Nikulin et al., 1997)	*Stachybotrys chartarum*	Satratoxins G+H	1.4×10^{-4}	214,286	*S. atra* s. 72 cultured on rice flour agar: "The spores of strain s. 72 contained satratoxins G and H, stachybotrylactone, and stachybotrylactam at 4 ng, 10 ng, 8 mg, and 2 mg/10^5 spores, respectively." Analytical method not specified.
(Ren et al., 1999)	*Aspergillus flavus*	Aflatoxin B_1	4.8×10^{-4}	62,500	NRRL-3251 cultured on glucose yeast extract agar—highest production of 8 strains evaluated. No significant mycotoxin production by any strain grown on building materials. Aflatoxin determined by TLC and HPLC

(*Continued*)

TABLE 6
(*Continued*)

Published source of mycotoxin data	Mold species	Mycotoxin	Maximum ng mycotoxin per spore	Minimum Spores/m^3 to reach CoNTC[a]	Comments
(Ren et al., 1999)	*Aspergillus flavus*	Aflatoxin B_1+B_2	6.2×10^{-4}	48,387	NRRL-3251 cultured on glucose yeast extract agar—highest production of 8 strains evaluated. No significant mycotoxin production by any strain grown on building material. Aflatoxin determined by TLC and HPLC
(Skaug et al., 2000)	*Penicillium verrucosum*	Ochratoxin A	7.0×10^{-4}	42,857	CBS 263.67 cultured on Sabouraud agar OTA determined by HPLC
(Yike et al., 2002)	*Stachybotrys chartarum*	Satratoxin G "equivalents"	1.0×10^{-3}	30,000	*S. chartarum* strain JS5817 cultured on potato dextrose agar. 1 pg SG equivalents/spore. Determined by the luciferase translation inhibition assay
(Størmer et al., 1998)	*Penicillium verrucosum*	Citrinin	4.1×10^{-3}	7,317	Highest value from 3 IBT strains: 5075, 5084, and 5258, each grown on MEA and SAB, range 1.4–4.1 pg/spore (4.1 pg/spore from IBT5075 on MEA). Determined by HPLC and mass spectroscopy

Note. CBS, Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; HPLC, high-performance liquid chromatography; IBT, IBT Culture Collection of Fungi, Mycology Group, BioCentrum-DTU, Technical University of Denmark, Lyngby, Denmark; MEA, malt extract agar; NRRL, Agricultural Research Service Culture Collection, National Center for Agricultural Utilization Research, U.S. Department of Agriculture, Peoria, IL; OTA, ochratoxin A; SAB, Saboraud agar; SRRC, Southern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, New Orleans, LA; TLC, thin layer chromatography; YES medium, yeast extract sucrose medium.

[a]Minimum spores/m^3 = 30 ng/m^3 (CoNTC)/maximum ng mycotoxin/spore.

Despite limited mycotoxin-specific exposure and toxicity data, there are published attempts to model human inhalation exposure to mycotoxins. Models based on spore doses all assume 100% bioavailability of any mycotoxins present, and all of these models ignore the clearance of deposited particulate matter and of metabolism and excretion of absorbed mycotoxins. Therefore, they provide an upper-bound perspective on potential doses. Burge (1996) used the published (Sorenson et al., 1987) concentration of satratoxin H in *Stachybotrys atra* spores to estimate the exposure duration required at various airborne spore concentrations for humans to inhale a cumulative 1-ng dose. That cumulative dose was not suggested to represent a toxic dose, but was chosen as an "extremely conservative" target. Calculated durations of exposure included 1100 d at 100 spores/m^3, 11 d at 10,000 spores/m^3, or 0.1 d at 1,000,000 spores/m^3. Kelman et al. (2004) extended the Burge (1996) model to estimate, for 8 mycotoxins or combinations of mycotoxins, the maximum 24-h dose that could be inhaled by a 78.1-kg adult man, assuming exposure to 200,000 spores/m^3 with each spore carrying the maximum published amount of those mycotoxins. Modeled airborne mycotoxin concentrations ranged from 0.2 to 96 ng/m^3. Comparison of the calculated dose to published toxicity data for the same mycotoxins showed effect doses to be orders of magnitude higher than the modeled maximum inhaled doses.

Using the published (Sorenson et al., 1987) concentration of satratoxin H per spore, the American College of Occupational and Environmental Medicine (ACOEM, 2003) estimated that 10^{10} spores/m^3 would be required to achieve 1 mg satratoxin/m^3, which was the no-effect concentration of T-2 toxin in 10-min rat inhalation exposures (mice and guinea pigs were similarly exposed but rats were the most sensitive species) (Creasia et al., 1987, 1990). ACOEM also modeled the spores per cubic meter concentrations that would deliver the same spore dose (spores/kg body weight) to a human as was delivered intratracheally to rats in a single-dose study (Rao et al., 2000), or intranasally to mice (cumulatively) in a 3-wk repeat-dose study (Nikulin et al., 1997). Infants, children, and adults were modeled separately. The no-effect spore dose of *S. chartarum* administered to rats was calculated to require a 24-h exposure

to 2.1×10^6 (infants) to 15.3×10^6 (adults) spores/m^3. The inflammatory but non-hemorrhagic cumulative spore dose of *S. chartarum* administered to mice was calculated to require 3 wk of continuous exposure (24 h/d, 7 d/wk) to 9.4×10^3 (infants) to 68×10^3 (adults) spores/m^3.

Miller et al. (2001) developed a risk assessment model based on lung surface area and the number of spores deposited there. Applying that model to the same rat single-dose study (Rao et al., 2000) used by ACOEM (2003), Miller et al. (2001) calculated that the no-effect dose of *S. chartarum* spores had been equivalent to 20 spores/cm^2 of rat lung surface area. In comparison, the model indicated that humans exposed to 1000 *S. chartarum* spores/m^3 for 1200 h (50 d of continuous exposure) would accumulate a total dose of 0.05 spores/cm^2 of lung surface.

None of these modeling efforts are adequate to provide a broad sense of the circumstances under which airborne mycotoxins might represent a human health hazard. It is reasonable to suspect that in occupational environments where dust and spore counts are high, inhaled doses of mycotoxins might be sufficient to produce or contribute to adverse health effects. For example, exposure to a tremorgenic mycotoxin was suspected when otherwise unexplained tremors developed in a 16-yr-old farm worker exposed to moldy silage; the condition resolved within a week (Gordon et al., 1993). Ochratoxin A was suspected as the cause of acute renal failure after a female farmer worked 8 h inside a granary containing wheat later determined to be contaminated with *Aspergillus ochraceous*. The condition resolved within 40 d (Di Paolo et al., 1993). There have also been suggestions that occupational exposure to aflatoxin-contaminated grain dust may pose a cancer risk for grain handlers (Autrup et al., 1991; Olsen et al., 1988).

Case series of organic dust toxic syndrome (ODTS) were reported in farmers exposed to high concentrations of agricultural dusts. ODTS is a self-limited condition characterized by a fever with chills, malaise, myalgia, headache, dyspnea, chest tightness, dry cough, and nausea (Seifert et al., 2003). ODTS does not have an immunologic basis and thereby is distinguished from hypersensitivity pneumonitis. ODTS was associated with exposures to agricultural dusts containing 10^9 to 10^{10} spores/m^3, whereas reference farms with no history of ODTS had spore counts of 10^8 spores/m^3 (Malmberg et al., 1993). Malmberg et al. (1993) associated ODTS with an estimated inhaled dose of 2.3×10^{10} spores/d, in contrast to an estimated spore dose of 2×10^8 spores/d on reference farms with no history of ODTS. The specific etiologic factors in ODTS are not known, but endotoxins from gram-negative bacteria, glucans from fungi, and mycotoxins are all suspected, alone or in combination with other components of agricultural dusts, e.g., plant debris, arthropod body parts, rodent and other excreta, and mineral dusts from the soil.

It is important to remember that the TTC and the CoNTC do not represent thresholds of adverse responses. Rather, they represent exposures that are lower, with a wide margin of safety, than the level at which any adverse response would be expected. The CoNTC as developed by Drew and Frangos (2007) was based on a daily dose of 0.01 μg/kg/d, derived from 50% of the TTC (1.5 μg/d) representing the U.S. FDA regulatory threshold for indirect food additives. The European Chemicals Bureau's Toxtree program (http://ecb.jrc.it/qsar/) categorizes each of the mycotoxins listed in Table 6 as Cramer class III, for which the TTC is 90 μg/d (Kroes et al., 2000; Munro et al., 1996a). Applying the mathematical model used by Drew and Frangos (2007), using the same default assumptions and basing the calculation on 45 μg/d (50% of the TTC), a Cramer-class III CoNTC would be 2.3 μg/m^3 (2,300 ng/m^3). This underscores the conservatism inherent in the 30 ng/m^3 CoNTC as originally proposed by Drew and Frangos (2007) and applied here.

It should also be noted that when Drew and Frangos (2007) proposed the CoNTC, they were able to test its validity by comparison with existing ambient air standards, with occupational exposure limits (scaled from 8 h/d 5 d/week to 24 h/d, 7 d/wk, and reduced by an additional 10-fold safety factor), and with guidelines for 1-h acute general population exposures. Each of those comparisons showed 30 ng/m^3 to be a protective screening level, whether for acute or chronic effects. Because no comparable database of human inhalation dose-response exists for mycotoxins, applicability of the CoNTC to inhaled mycotoxins cannot be similarly validated.

Inherent in using the CoNTC as an index of potential hazard from mycotoxins is the assumption that the TTC are applicable; no mycotoxins were included in the databases originally used to establish the Cramer classes (Cramer et al., 1978) or to establish TTCs (Munro et al., 1996a, 1996b). Mycotoxins nevertheless consist of the same molecular structures and functional groups upon which the Cramer classes are based. There is no obvious reason to predict substantial differences in structure–function relationships based on those functional groups being present in mycotoxins versus nonmycotoxins. Even if, as for OP esters, a robust dose-response database for mycotoxins were found to argue for a TTC lower than that for Cramer class III, the regulatory threshold for indirect food additives from which the CoNTC is derived likely would prove to be protective, as it was found to be for OP esters.

When a decision tree was developed to guide implementation of TTCs in food safety evaluations (Kroes et al., 2004), the Expert Group excluded aflatoxin-like compounds and recommended safety assessments be based on compound-specific toxicity data. Guidance levels for aflatoxins (and other mycotoxins) in foods are established in almost 100 nations (FAO, 2004). The only guidance level that is lower than the U.S. FDA regulatory threshold (0.5 ppb) is that for aflatoxin M_1 in milk (0.05 ppb) in the European Union. Guidance levels for total aflatoxins all are severalfold greater than 0.5 ppb, and some guidance levels for other mycotoxins exceed the TTC for Cramer class I (Tables 1 and 2). Guidance levels for foods were not proposed, but the European Union (EU) Scientific Committee on Food established tolerable daily intakes (TDI)

for fumonisin B_1 (2 μg/kg body weight/d) and deoxynivalenol (1 μg/kg body weight/d), and tentative TDI for nivalenol (0.7 μg/kg body weight/d) and combined T-2 and HT-2 toxins (0.06 μg/kg body weight/d) (Scientific Committee on Food, 2000, 2002). For a 60-kg person, the lowest of these TDI (0.06 μg/kg body weight/d) is 2.4-fold higher than intake (0.025 μg/kg body weight/d) assumed at the U.S. FDA regulatory threshold (see Table 1). Data are not available to validate route-to-route (dietary-to-inhalation) extrapolation. Nevertheless, in the absence of contrary evidence it seems reasonable to employ the CoNTC for screening-level mycotoxin safety assessments.

Table 3 illustrates the range of airborne concentrations of mold spores and total dust that may be encountered in agricultural and some other occupational environments. Peak concentrations of 5×10^9 to 24×10^9 spores/m^3 were reported (Halstensen et al., 2007; Palmgren et al., 1986), and 10^6 to 10^7 spores/m^3 are frequently observed. As seen in Table 4, some agricultural dusts contained measured amounts of mycotoxins sufficient to exceed the CoNTC at the 15 mg/m^3 OSHA PEL for total dust.

Table 5 shows that aflatoxin concentrations above the CoNTC were directly measured in agricultural environments. The implications of results in Table 5 from indoor air samples are unclear because unvalidated, nonspecific analytical methods were used. Nevertheless, the highest reported indoor air concentration was not meaningfully above the CoNTC.

More extensive data are available and summarized in Table 6 on the highest measured amounts of various mycotoxins in fungal spores. These data indicate that even for spores that contain those highest-reported levels of mycotoxin, airborne spore concentrations in the range reported in agricultural environments are required to attain the CoNTC. It is not known whether these mycotoxin per-spore maxima are attained by molds growing in natural environments.

Exposures in the most extreme of the working conditions described here are sufficiently high that airborne mycotoxin concentrations in excess of 30 ng/m^3 exist. Because of the conservatism inherent in the CoNTC, these actual or potential exposures above the CoNTC do not necessarily predict a mycotoxin-induced adverse health effect. However, they do call for improved dust suppression and suggest a need for more detailed exposure assessments.

In agricultural and similar environments where airborne dust or spore concentrations suggest the potential to exceed the CoNTC, direct determinations of airborne mycotoxins are needed to establish which mycotoxins are most prevalent and at what concentrations. For the mycotoxins most in excess or most frequently in excess of the CoNTC, reliable risk characterizations will require improved toxicity profiles with mycotoxin-specific exposure-response data and, potentially, investigation of how bioavailable mycotoxins in spores, fungal fragments, and dusts may be. On the other hand, the data compiled here suggest that outside of these dusty environments, for example, in the built indoor environment where dust and spore concentrations are orders of magnitude lower, exposures to mycotoxins are below 30 ng/m^3, the concentration of no toxicologic concern.

REFERENCES

ACOEM. 2003. Evidence-based statement—Adverse human health effects associated with molds in the indoor environment. *J. Occup. Environ. Med.* 45:470–478.

Autrup, J. L., Schmidt, J., Seremet, T., and Autrup, H. 1991. Determination of exposure to aflatoxins among Danish workers in animal-feed production through the analysis of aflatoxin B1 adducts to serum albumin. *Scand. J. Work Environ. Health* 17:436–440.

Ball, D., Blanchard, J., Jacobson-Kram, D., McClellan, R. O., McGovern, T., Norwood, D. L., Vogel, W., Wolff, R., and Nagao, L. 2007. Development of safety qualification thresholds and their use in orally inhaled and nasal drug product evaluation. *Toxicol. Sci.* 97:226–236.

Baruah, H. K. 1961. The air spora of a cowshed. *J. Gen. Microbiol.* 5:483–491.

Blackburn, K., Stickney, J. A., Carlson-Lynch, H. L., McGinnis, P. M., Chappell, L., and Felter, S. P. 2005. Application of the threshold of toxicological concern approach to ingredients in personal and household care products. *Regul. Toxicol. Pharmacol.* 43:249–259.

Bloom, E., Bal, K., Nyman, E., Must, A., and Larsson, L. 2007. Mass spectrometry-based strategy for direct detection and quantification of some mycotoxins produced by *Stachybotrys* and *Aspergillus* spp. in indoor environments. *Appl. Environ. Microbiol.* 73:4211–4217.

Brasel, T. L., Martin, J. M., Carriker, C. G., Wilson, S. C., and Straus, D. C. 2005. Detection of airborne *Stachybotrys chartarum* macrocyclic trichothecene mycotoxins in the indoor environment. *Appl. Environ. Microbiol.* 71:7376–7388.

Burg, W., Shotwell, O. L., and Saltzman, B. E. 1981. Measurements of airborne aflatoxins during the handling of contaminated corn. *Am. Ind. Hyg. Assoc. J.* 42:1–11.

Burg, W. R., Shotwell, O. L., and Saltzman, B. E. 1982. Measurement of airborne aflatoxins during the handling of 1979 contaminated corn. *Am. Ind. Hyg. Assoc. J.* 43:580–586.

Burge, H. A. 1996. Health effects of biological contaminants. In *Indoor air and human health*, eds. R. B, Gammage and B. A. Berven, pp. 171–178. Boca Raton, FL: CRC Press.

Chapman, J. A. 2003. *Stachybotrys chartarum* (*chartarum=atra=alternans*) and other problems caused by allergenic fungi. *Allergy Asthma Proc.* 24:1–7.

Cho, S.-H., Seo, S.-C., Schmechel, D., Grinshpun, S. A., and Reponen, T. 2005. Aerodynamic characteristics and respiratory deposition of fungal fragments. *Atmos. Environ.* 39:5454–5465.

Cramer, G. M., Ford, R. A., and Hall, R. L. 1978. Estimation of toxic hazard—A decision tree approach. *Food Cosmet. Toxicol.* 16:255–276.

Creasia, D. A., Thurman, J. D., Jones, L. J., Nealley, M. L., York, C. G., Wannemacher, R. W., Jr., and Bunner, D. L. 1987. Acute inhalation toxicity of T-2 mycotoxin in mice. *Fundam. Appl. Toxicol.* 8:230–235.

Creasia, D. A., Thurman, J. D., Wannemacher, R. W., Jr., and Bunner, D. L. 1990. Acute inhalation toxicity of T-2 mycotoxin in the rat and guinea pig. *Fundam. Appl. Toxicol.* 14:54–59.

Darke, C. S., Knowelden, J., Lacey, J., and Ward, A. M. 1976. Respiratory disease of workers harvesting grain. *Thorax* 31:294–302.

Di Paolo, N., Guarnieri, A., Loi, F., Sacchi, G., Mangiarotti, A. M., and Di Paolo, M. 1993. Acute renal failure from inhalation of mycotoxins. *Nephron* 64:621–625.

Dolan, D. G., Naumann, B. D., Sargent, E. V., Maier, A., and Dourson, M. 2005. Application of the threshold of toxicological concern concept to pharmaceutical manufacturing operations. *Regul. Toxicol Pharmacol* 43:1–9.

Drew, R., and Frangos, J. 2007. The concentration of no toxicological concern (CoNTC): A risk assessment screening tool for air toxics. *J. Toxicol. Environ. Health A* 70:1584–1593.

Duchaine, C., Meriaux, A., Thorne, P. S., and Cormier, Y. 2000. Assessment of particulates and bioaerosols in eastern Canadian sawmills. *Am. Ind. Hyg. Assoc. J.* 61:727–732.

Durand, K. T., Muilenberg, M. L., Burge, H. A., and Seixas, N. S. 2002. Effect of sampling time on the culturability of airborne fungi and bacteria sampled by filtration. *Ann. Occup. Hyg.* 46:113–118.

Eduard, W., Douwes, J., Mehl, R., Heederik, D., and Melbostad, E. 2001. Short term exposure to airborne microbial agents during farm work: Exposure-response relations with eye and respiratory symptoms. *Occup. Environ. Med.* 58:113–118.

FAO. 2004. *Worldwide regulations for mycotoxins in food and feed in 2003. FAO Food and Nutrition Papers 81*. ftp://ftp.fao.org/docrep/fao/007/y5499e/y5499e00.pdf

FDA. 1995. Food additives—Threshold of regulation for substances used in food-contact articles. *Fed. Reg.* 60:36582–36596.

Frawley, J. P. 1967. Scientific evidence and common sense as a basis for food-packaging regulations. *Food Cosmet. Toxicol.* 5:293–308.

Gordon, K. E., Masotti, R. E., and Waddell, W. R. 1993. Tremorgenic encephalopathy: A role of mycotoxins in the production of CNS disease in humans? *Can. J. Neurol. Sci.* 20:237–239.

Górny, R. L., Reponen, T., Grinshpun, S. A., and Willeke, K. 2001. Source strength of fungal spore aerosolization from moldy building material. *Atmos. Environ.* 35:4853–4862.

Górny, R. L., Reponen, T., Willeke, K., Schmechel, D., Robine, E., Boissier, M., and Grinshpun, S. A. 2002. Fungal fragments as indoor air biocontaminants. *Appl. Environ. Microbiol.* 68:3522–3531.

Halstensen, A. S., Nordby, K. C., Wouters, I. M., and Eduard, W. 2007. Determinants of microbial exposure in grain farming. *Ann. Occup. Hyg.* 51:581–592.

Heldal, K. K., and Eduard, W. 2004. Associations between acute symptoms and bioaerosol exposure during the collection of household waste. *Am. J. Ind. Med.* 46:253–260.

Heldal, K. K., Halstensen, A. S., Thorn, J., Djupesland, P., Wouters, I., Eduard, W., and Halstensen, T. S. 2003. Upper airway inflammation in waste handlers exposed to bioaerosols. *Occup. Environ. Med.* 60:444–450.

Herr, C. E., zur Nieden, A., Jankofsky, M., Stilianakis N. I., Boedeker, R. H., and Eikmann, T. F. 2003. Effects of bioaerosol polluted outdoor air on airways of residents: A cross sectional study. *Occup. Environ. Med.* 60:336–342.

Humfrey, C. D. 2007. Risk assessment below the limits of detection? Recent developments in the risk assessment of potentially genotoxic impurities in pharmaceutical drug substances. *Toxicol. Sci.* 100:24–28.

Iavicoli, I., Brera, C., Carelli, G., Caputi, R., Marinaccio, A., and Miraglia, M. 2002. External and internal dose in subjects occupationally exposed to ochratoxin A. *Int. Arch. Occup. Environ. Health* 75:381–386.

Kelman, B. J., Robbins, C. A., Swenson, L. J., and Hardin, B. D. 2004. Risk from inhaled mycotoxins in indoor office and residential environments. *Int. J. Toxicol.* 23:3–10.

Kroes, R., Galli, C., Munro, I., Schilter, B., Tran, L., Walker, R., and Wurtzen, G. 2000. Threshold of toxicological concern for chemical substances present in the diet: A practical tool for assessing the need for toxicity testing. *Food Chem. Toxicol.* 38:255–312.

Kroes, R., Kleiner, J., and Renwick, A. 2005. The threshold of toxicological concern concept in risk assessment. *Toxicol. Sci.* 86:226–230.

Kroes, R., and Kozianowski, G. 2002. Threshold of toxicological concern (TTC) in food safety assessment. *Toxicol. Lett.* 127:43–46.

Kroes, R., Renwick, A. G., Cheeseman, M., Kleiner, J., Mangelsdorf, I., Piersma, A., Schilter, B., Schlatter, J., van Schothorst, F., Vos, J. G., and Wurtzen, G. 2004. Structure-based thresholds of toxicological concern (TTC): Guidance for application to substances present at low levels in the diet. *Food Chem. Toxicol.* 42:65–83.

Kroes, R., Renwick, A. G., Feron, V., Galli,C. L., Gibney, M., Greim, H., Guy, R. H., Lhuguenot, J. C., and van de Sandt, J. J. 2007. Application of the threshold of toxicological concern (TTC) to the safety evaluation of cosmetic ingredients. *Food Chem. Toxicol.* 45:2533–2562.

Land, C. J., Lundstrom, H., and Werner, S. 1993. Production of tremorgenic mycotoxins by isolates of *Aspergillus fumigatus* from sawmills in Sweden. *Mycopathologia* 124:87–93.

Land, C. J., Rask-Andersen, A., Lundström, H., Werner, S., and Bardage, S. 1994. Tremorgenic mycotoxins in conidia of *Aspergillus fumigatus*. In *Health implications of fungi in indoor air environments*, eds. R. A. Samson, B. Flannigan, M. E. Flannigan, A. P. Verhoeff, O. C. G. Adan, and E. S. Hoekstra, pp. 307–315. Amsterdam: Elsevier/North Holland Biomedical Press.

Lappalainen, S., Nikulin, M., Berg, S., Parikka, P., Hintikka, E.-L., and Pasanen, A.-L. 1996. *Fusarium* toxins and fungi associated with handling of grain on eight Finnish farms. *Atmos. Environ.* 30:3059–3065.

Lee, S. A., Adhikari, A., Grinshpun, S. A., McKay, R., Shukla, R., and Reponen, T. 2006. Personal exposure to airborne dust and microorganisms in agricultural environments. *J. Occup. Environ. Hyg.* 3:118–130.

Malmberg, P., Rask-Andersen, A., and Rosenhall, L. 1993. Exposure to microorganisms associated with allergic alveolitis and febrile reactions to mold dust in farmers. *Chest* 103:1202–1209.

May, J. J., Pratt, D. S., Stallones, L., Morey, P. R., Olenchock, S. A., and Deep, I. W. 1986. A study of silo unloading—The work environment and its physiologic effects. *Am. J. Ind. Med.* 10:318.

Melbostad, E., and Eduard, W. 2001. Organic dust-related respiratory and eye irritation in Norwegian farmers. *Am. J. Ind. Med.* 39:209–217.

Miller, R. V., Martinez-Miller, C., and Bolin, V. 2001. A novel risk assessment model for the evaluation of fungal exposure in indoor environments. In *Mycotoxins and phycotoxins in perspective at the turn of the millenium. Proceedings of the Xth International IUPAC Symposium on Mycotoxins and Phycotoxins, Guaruja, Brazil, 21–25 May 2000*, eds. W. J. de Koe, R. A. Samson, H. P. van Egmond, J. Gilbert, and M. Sabino, pp. 123–131.

Morey, P. R. 1990. Practical aspects of sampling for organic dusts and microorganisms. *Am. J. Ind. Med.* 18:273–278.

Müller, L., Mauthe, R. J., Riley, C. M., Andino, M. M., Antonis, D. D., Beels, C., DeGeorge, J., De Knaep, A. G., Ellison, D., Fagerland, J. A., Frank, R., Fritschel, B., Galloway, S., Harpur, E., Humfrey, C. D., Jacks, A. S., Jagota, N., Mackinnon, J., Mohan, G., Ness, D. K., O'Donovan, M. R., Smith, M. D., Vudathala, G., and Yotti, L. 2006. A rationale for determining, testing, and controlling specific impurities in pharmaceuticals that possess potential for genotoxicity. *Regul. Toxicol. Pharmacol.* 44:198–211.

Munro, I. C. 1990. Safety assessment procedures for indirect food additives: an overview. Report of a workshop. *Regul. Toxicol. Pharmacol.* 12:2–12.

Munro, I. C., Ford, R. A., Kennepohl, E., and Sprenger, J. G. 1996a. Correlation of structural class with no-observed-effect levels: A proposal for establishing a threshold of concern. *Food Chem. Toxicol.* 34:829–867.

Munro, I. C., Ford, R. A., Kennepohl, E., and Sprenger, J. G. 1996b. Thresholds of toxicological concern based on structure–activity relationships. *Drug Metab. Rev.* 28:209–217.

Munro, I. C., Kennepohl, E., and Kroes, R. 1999. A procedure for the safety evaluation of flavouring substances. Joint FAO/WHO Expert Committee on Food Additives. *Food Chem. Toxicol.* 37:207–232.

Munro, I. C., Renwick, A. G., and Danielewska-Nikiel, B. 2008. The Threshold of Toxicological Concern (TTC) in risk assessment. *Toxicol. Lett.* 180:151–156.

Nikulin, M., Reijula, K., Jarvis, B. B., Veijalainen, P., and Hintikka, E. L. 1997. Effects of intranasal exposure to spores of *Stachybotrys atra* in mice. *Fundam. Appl. Toxicol.* 35:182–188.

Nordby, K. C., Halstensen, A. S., Elen, O., Clasen, P. E., Langseth, W., Kristensen, P., and Eduard, W. 2004. Trichothecene mycotoxins and their determinants in settled dust related to grain production. *Ann. Agric. Environ. Med.* 11:75–83.

Olsen, J. H., Dragsted, L., and Autrup, H. 1988. Cancer risk and occupational exposure to aflatoxins in Denmark. *Br. J. Cancer* 58:392–396.

Palmgren, M. S., and Lee, L. S. 1986. Separation of mycotoxin-containing sources in grain dust and determination of their mycotoxin potential. *Environ. Health Perspect.* 66:105–108.

Palmgren, U., Strom, G., Blomquist, G., and Malmberg, P. 1986. Collection of airborne micro-organisms on Nuclepore filters, estimation and analysis—CAMNEA method. *J. Appl. Bacteriol.* 61:401–406.

Patlewicz, G., Jeliazkova, N., Safford, R. J., Worth, A. P., and Aleksiev, B. 2008. An evaluation of the implementation of the Cramer classification scheme in the Toxtree software. *SAR QSAR Environ. Res.* 19:495–524.

Rao, C. Y. 2001. Toxigenic fungi in the indoor environment. In Indoor air quality handbook, eds. J. D. Spengler, J. M. Samet, and J. F. McCarthy, pp. 46.1–46.17. New York: McGraw Hill.

Rao, C. Y., Brain, J. D., and Burge, H. A. 2000. Reduction of pulmonary toxicity of *Stachybotrys chartarum* spores by methanol extraction of mycotoxins. *Appl. Environ. Microbiol.* 66:2817–2821.

Rao, C. Y., Burge, H. A., and Chang, J. C. 1996. Review of quantitative standards and guidelines for fungi in indoor air. *J. Air Waste Manage. Assoc.* 46:899–908.

Ren, P., Ahearn, D. G., and Crow, S. A. 1999. Comparative study of *Aspergillus* mycotoxin produced on enriched media and construction material. *J. Ind. Microbiol. Biotechnol.* 21:209–213.

Renwick, A. G. 2005. Structure-based thresholds of toxicological concern—Guidance for application to substances present at low levels in the diet. *Toxicol. Appl. Pharmacol.* 207:585–591.

Safford, R. J. 2008. The dermal sensitisation threshold—A TTC approach for allergic contact dermatitis. *Regul. Toxicol. Pharmacol.* 51:195–200.

Scientific Committee on Food. 2000. Opinion of the Scientific Committee on Food on Fusarium Toxins Part 3: Fumonisin B_1 (FB_1). in European Commission Health & Consumer Protection Directorate-General Directorate C—Scientific Opinions C3—Management of scientific committees II; Scientific co-operation and networks, pp. 1–33. Brussels, Belgium.

Scientific Committee on Food. 2002. Opinion of the Scientific Committee on Food on Fusarium toxins. Part 6: Group evaluation of T-2 toxin, HT-2 toxin, nivalenol and deoxynivalenol. in European Commission Health & Consumer Protection Directorate—General Directorate C—Scientific Opinions C2—Management of scientific committees; Scientific co-operation and networks, pp. 1–12. Brussels, Belgium.

Seifert, S. A., von Essen, S., Jacobitz, K., Crouch, R., and Lintner, C. P. 2003. Organic dust toxic syndrome: A review. *J. Toxicol. Clin. Toxicol.* 41:185–193.

Selim, M. I., Juchems, A. M., and Popendorf, W. 1998. Assessing airborne aflatoxin B_1 during on-farm grain handling activities. *Am. Ind. Hyg. Assoc. J.* 59:252–256.

Skaug, M. A., Eduard, W., and Størmer, F. C. 2000. Ochratoxin A in airborne dust and fungal conidia. *Mycopathologia* 151:93–98.

Sorenson, W. G., Frazer, D. G., Jarvis, B. B., Simpson, J., and Robinson, V. A. 1987. Trichothecene mycotoxins in aerosolized conidia of *Stachybotrys atra*. *Appl. Environ. Microbiol.* 53:1370–1375.

Sorenson, W. G., Jones, W., Simpson, J., and Davidson, J. I. 1984. Aflatoxin in respirable airborne peanut dust. *J. Toxicol. Environ. Health* 14:525–533.

Størmer, F. C., Sandven, P., Huitfeldt. H. S., Eduard. W., and Skogstad. A. 1998. Does the mycotoxin citrinin function as a sun protectant in conidia from *Penicillium verrucosum*. *Mycopathologia* 142:43–47.

Todd, B. E., and Buchan, R. M. 2002. Total dust, respirable dust, and microflora toxin concentrations in Colorado corn storage facilities. *Appl. Occup. Environ. Hyg.* 17:411–415.

Tuomi, T., Reijula, K., Johnsson, T., Hemminki, K., Hintikka, E. L., Lindroos, O., Kalso, S., Koukila-Kahkola, P., Mussalo-Rauhamaa, H., and Haahtela, T. 2000. Mycotoxins in crude building materials from water-damaged buildings. *Appl. Environ. Microbiol.* 66:1899–1904.

Wilson, R. M., Sigal, E. A., Bacigalupo, C. M., Willes, R. F., and Munro, I. C. 2000. Derivation of risk management criteria for chemicals of unknown potency at contaminated sites. *Hum. Ecol. Risk Assess.* 6:131–139.

Yike, I., Allan, T., Sorenson, W. G., and Dearborn, D. G. 1999. Highly sensitive protein translation assay for trichothecene toxicity in airborne particulates: Comparison with cytotoxicity assays. *Appl. Environ. Microbiol.* 65:88–94.

Yike, I., Miller, M. J., Sorenson, W. G., Walenga, R., Tomashefski, J. F., and Dearborn, D. G. 2002. Infant animal model of pulmonary mycotoxicosis induced by *Stachybotrys chartarum*. *Mycopathologia* 154:139–152.