Chapter 10
Microbial Secondary Metabolites and Knowledge on Inhalation Effects

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Abstract Microbial secondary metabolites include compounds produced during the growth of both fungi and bacteria. These compounds are present in workplaces and indoor environments, although the concentrations of single toxins in the air are typically low. Inhalation is considered to be the most significant route of exposure for microbial secondary metabolites in indoor environments although exposure to microbial toxins may happen also via alimentary or dermal route. Inhalation effects of microbial secondary metabolites have been studied experimentally in vivo in animal models, mainly in rodents. In vitro studies with cells of respiratory system and ex vivo cultured tissues have elucidated the mechanisms of action for the most common toxins. However, there are only few epidemiological studies on health effects of mycotoxin exposure, and often the studies are limited by exposure assessment based on single compounds or surrogates of mycotoxin exposure. We summarize here studies on the inhalation effects of microbial toxins showing a wide variety of adverse health effects which are not limited to the respiratory system, and identify the knowledge gaps where future research efforts should be targeted.

Keywords Microbial secondary metabolites · mycotoxin · inhalation · toxicity

10.1 Introduction

Mycotoxins, i.e. toxic secondary metabolites of fungi have been recognized as a health concern since early 1960s, after the revelation that aflatoxin-contaminated grain was the reason for mass killings of poultry (Forgacs, 1962). The number of

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identified mycotoxins (~400 individual compounds) is only a fraction of the total number of toxic metabolites of fungi in our environment (Council for Agricultural Science and Technology, 2003; Täubel and Hyvärinen, 2015). In addition to fungi, also bacteria are able to produce toxic compounds during their growth (Täubel et al., 2011), which increases the number of toxic microbial metabolites possibly relevant to human exposure in the order of thousands. The main exposure route for mycotoxins is alimentary, e.g. eating contaminated foods, which directed the early research efforts into studying the effects of fungi typically present in foods or feeds, and oral exposure as a point of entry. Also dermal or mucosal absorption is possible, but to a lesser extent (Boonen et al., 2012; Pinton and Oswald, 2014). Exposure through respiratory route did not appear to be as relevant, as the majority of mycotoxins are nonvolatile. However, it was soon realized that inhalation is also a major route of exposure to mycotoxins. Not only were mycotoxins found from airborne dust (Croft et al., 1986; Flannigan, 1987), but the spores and fragments of microbial growth were shown to carry mycotoxins along them to the lungs (Brasel et al., 2005).

As a portal of entry for inhaled air, the nasal airway is a primary target for many inhaled toxicants (Harkema et al., 2006). The surface epithelium lining is often the first tissue in the nose to be directly injured, for example, by spores or mycotoxins of *Stachybotrys chartarum* (Pestka et al., 2008). Also microbial volatile organic compounds (MVOCs) are known to cause eye and upper-airway irritation in experimental exposure studies both in animals and humans (Korpi et al., 2009). Even if the toxins are nonvolatile, the adverse effect of mycotoxins can be carried by spores, as was seen when studying the pulmonary toxicity of intranasal or intrathacheal exposure to spores of *S. chartarum* in mice and rats (Lichtenstein et al., 2010; Pestka et al., 2008; Yike and Dearborn, 2004; Yike et al., 2005). The effect of inhalation exposure can be further aggravated by the presence and persistence of structural components in the lungs, leading to high concentrations of toxins within spore's immediate microenvironment (Carey et al., 2012). Comparison of intranasal and oral routes of exposure to mycotoxin deoxynivalenol (DON) in mice showed clearly higher plasma and tissue concentrations, as well as higher mRNA expressions of proinflammatory cytokines in internal organs following intranasal exposure (Amuzie et al., 2008). Accordingly, acute toxicity of the trichothecene mycotoxin T-2 in mice, rats and guinea pigs has been shown to be higher after inhalation exposure than when delivered by other routes (Creasia et al., 1987; Creasia et al., 1990). Considering that the lungs are a likely entry point both for nonvolatile and volatile compounds, inhalation is arguably the most significant route of exposure for microbial secondary metabolites in indoor environments (Fig. 10.1).

First studies on inhalation exposure to mycotoxins appeared in the late 1980s, describing the acute effects of trichothecenes produced by *Stachybotrys* (Croft et al., 1986). In the early years, the research on inhalation effects focused on mycotoxins that originated from molds thriving in high water activity such as aflatoxin B1 (AFB1) produced by e.g. *Aspergillus flavus* and satratoxins from *Stachybotrys chartarum* (Bitnun and Nosal, 1999; Hodgson et al., 1998). Originally the data on mycotoxin concentrations were largely from the workplaces

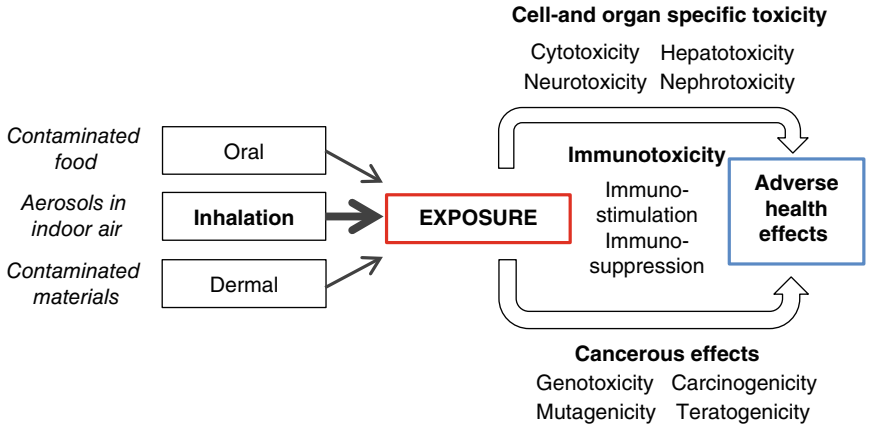

Fig. 10.1 Exposure to mycotoxins from various sources and some of the mechanisms and toxic properties leading to adverse health effects

that are routinely monitored for contamination such as factories processing grain, corn, peanut or malt. The most abundant mycotoxins found in the air of these workplaces are AFB1, DON and ochratoxin A (OTA) (Fromme et al., 2016). In addition of AFB1, DON and OTA, fumonisin and zearalenone are also agriculturally important mycotoxins with worldwide distribution (Jarvis and Miller, 2005; Miller and McMullin, 2014). Later, mycotoxins have been detected basically from all workplaces where organic material is handled including greenhouses, pigsties, cowsheds and waste treatment facilities (Degen, 2011; Mayer et al., 2008). In addition to the occupational exposure in industrial settings, the mycotoxin exposure in moisture damaged indoor environments has been a target of interest since 1980s, brought forward by the extensive media attention on cases of proposed intoxications with toxic secondary metabolites of *Stachybotrys chartarum* (Nevalainen et al., 2015). However, there is still a lack of exposure data, as the airborne mycotoxin concentrations are rarely measured, let alone associated with clinical health data. The challenge of the future studies is a better characterization of exposure to low concentrations of multiple toxins (Miller et al., 2010).

10.2 Toxicological Models of Respiratory System

Throughout the history of inhalation toxicology, small laboratory animals such as mice, rats, rabbits and guinea pigs have been used as model systems for pulmonary toxicity. In the case of a systemic effect to an inhaled toxicant, animal model is the only available approach for studying the effects in a controlled environment. In experimental conditions the most natural way of introducing mycotoxins into the lungs of laboratory animals is via inhalation (whole body exposure). However, inhalation exposure is restricted by the difficulties in assuring the actual dose in

the lungs of each animal as well as the need to use expensive equipment, special expertise and large quantities of the tested materials. For these reasons, intratracheal or intranasal instillation has become widely used for respiratory toxicity studies (Driscoll et al., 2000; Hasegawa-Baba et al., 2014; Islam et al., 2006, 2007). Rodent airways, however, differ from those of human and other primates both structurally and functionally, which has motivated the development of nonhuman primate models in order to better extrapolate the results to humans (Carey et al., 2012).

With an increasing interest and legislation to reduce the use of animals in research (EU, 2003; REACH, 2006), animal models are increasingly being replaced with in vitro models using human cells or tissues. In addition to ethical issues, in vitro toxicity testing appeals with the associated low cost, high throughput and the possibility to target a specific cell type in a defined experimental setup. The function of the respiratory epithelium as a first contact point for airborne exposure agents makes it an appealing target for toxicological studies aiming to model the effects of inhaled substances. The in vitro respiratory platforms include carcinoma-derived and virus-transformed continuous cell lines, primary cells and ex vivo models. Examples of cell lines typically used in inhalation toxicology are carcinoma-derived lung epithelial or bronchial cells (e.g. A549 and Calu-3) and immortalized human epithelial or bronchial cells (e.g. BEAS-2B and 16HBE140) (Prytherch and BéruBé, 2014). The next step from monolayers of transformed cell lines would be to utilize more complicated cell models, where e.g. macrophages are cultured together with lung epithelia to mimic the components of immune system operational in the lungs. The co-culture models for alimentary exposure to mycotoxins are already in use, as was shown in a study of modulation of inflammatory response by OTA in a co-culture of intestinal and liver cells (González-Arias et al., 2015). However, for inhalation exposure to mycotoxins the studies using co-culture models of transformed cells are still lacking.

Primary cells isolated from nasal and bronchial epithelium or from the alveolar level of the lungs have been used in inhalation toxicology for producing cultures of respiratory tissue with close to realistic morphological and physiological properties (Prytherch et al., 2011). For example the impairment of motile and chemosensory functions due to aflatoxin exposure has been demonstrated with cultures of primary human sinonasal and bronchial cells (Lee et al., 2016). Cultures of primary respiratory cells have shown also the ability of airway epithelium to detoxify both AFB1 and sterigmatocystin (Cabaret et al., 2010; Van Vleet et al., 2001). Generating respiratory tissue by culturing embryonic or induced pluripotent stem cells is possible as well, but achieving accurate characteristics and homogenous bronchial epithelium still needs further development (McIntyre et al., 2015). Alternatively, complete sections of respiratory tissue can be extracted and cultured ex vivo, maintaining the complexity and function of an intact lung. Ex vivo experiments with rat tracheas have elucidated e.g. the vasoconstrictive abilities of OTA (Chatopadhyay et al., 2014) and ciliostatic abilities of several metabolites of indoor fungi (Piecková and Kunová, 2002). However, the life span of current ex vivo models is very limited, and similarly with cultures of primary cells, also cultured tissues are donor specific and thus vary in their responses.

10.3 Microbial Secondary Metabolites in Indoor Environments

Microbial secondary metabolites have been detected from water-damaged indoor environments both in private residents and public buildings, although the concentrations of single toxins are typically low and the diversity high (Bloom et al., 2009; Cai et al., 2011; Kirjavainen et al., 2015; Peitzsch et al., 2012; Polizzi et al., 2009; Täubel et al., 2011; Tuomi et al., 2000). The most widely studied mycotoxins detected indoors are aflatoxins (sterigmatocystin) (Bloom et al., 2009), OTA (Hope and Hope, 2012) and trichothecene mycotoxins (T2, DON, satratoxins, verrucarin) (Pestka et al., 2008; Straus, 2009).

Aflatoxins, especially AFB1, are known liver carcinogens, but they can also exert their effects on the lungs. The mechanism behind its carcinogenicity in lung is suggested to be oxidative DNA damage (Guindon-Kezis et al., 2014). The high local bioactivation and neuronal transport to the olfactory bulb seen after intranasal administration of AFB1 indicates a risk of tumorigenesis in nasal mucosa after high local exposure (Larsson and Tjälve, 2000). Aerosol exposures of animals to AFB1 have resulted in an increase of lymphatic leukemia in mice (Louria et al., 1974), genotoxic effects in rats (Zarba et al., 1992) and suppression of pulmonary and systemic host defenses in rats and mice (Jakab et al., 1994). Along other mycotoxins, also aflatoxins have been detected in patients with health problems related to exposure to water damaged indoor environment (Trasher et al., 2012; Brewer et al., 2013b). In the industrial setting, the inhalation exposure to aflatoxins has been linked to cancer incidence and pulmonary disorders (Dvorácková and Pichová, 1986; Hayes et al., 1984). Elevated levels of AFB1 were also measured in bronchoalveolar lavage and serum samples of workers in food-grain handling in India, where about 50% of all food-grain workers had chronic respiratory symptoms (Malik et al., 2014). A case-control study by Lai et al., 2014 (Lai et al., 2014) showed an association between the inhalation exposure to AFB1 and the risk of hepatocellular cancer among sugar and papermaking factory workers. Thus, the inhalation of aflatoxins represents an occupational risk factor.

OTA is one of the most important and deleterious mycotoxins contaminating foodstuff, leading to exposure also via inhalation. OTA is thought to be carcinogenic, teratogenic, immunotoxic, hepatotoxic and neurotoxic, but the detailed mechanisms of its toxicity are not known (Hope and Hope, 2012; Kőszegi and Poór, 2016; Malir et al., 2016). Nevertheless, inhibition of protein synthesis and energy production, induction of oxidative stress, DNA adduct formation as well as apoptosis/necrosis and cell cycle arrest are suggested to be involved in its toxic action (Corcuera et al., 2015; Vettorazzi et al., 2013). Elevated levels of OTA have been detected in urine of patients exposed to toxins in water-damaged buildings (Hooper et al., 2009) as well as in workers exposed to airborne dust originating from the processing of contaminated foods (Iavicoli et al., 2002). Inhalation of OTA has been linked to acute renal failure and respiratory distress in workers exposed to Aspergillus-producers of OTA in a granary (Di Paolo et al., 1994). Furthermore, OTA has been found in 22% of sinonasal tissue and mucus

of chronic rhinosinusitis patients, and 83% of the urine of patients suffering from chronic fatigue syndrome (CFS) (Brewer et al., 2013b).

Trichothecenes are a group of over 200 structurally related mycotoxins produced by various species of e.g. *Fusarium, Myrothecium* and *Stachybotrys*. It has been suggested that the toxicity of trichothecenes might be a reason for many of the adverse effects of *Stachybotrys chartarum*, an infamous black mold suggested to contribute to damp building related illnesses (Pestka et al., 2008). Macrocyclic trichothecenes have been detected in patients with health problems related to mold contaminated buildings in several studies (Brewer et al., 2013b; Croft et al., 2002; Dennis et al., 2009; Rea et al., 2003, Trasher et al., 2012). The toxic potencies of different inhaled mycotoxins have been compared in hamster lung cells in vitro, listing the T-2 and HT-2 toxins as the most cytotoxic mycotoxins followed by *Fusarium* toxins DON, beauvericin, and enniatin B (Behm et al., 2012). One possible mechanism for adverse health effects of trichothecenes is the impairment of host defense, as the immune system is extremely sensitive to their effects (Pestka et al., 2008; Pestka, 2010a). The effect may be bipolar, where low concentrations cause immunostimulation and high concentrations result in immunosuppression, which was shown in human immune cells in vitro (Pestka et al., 2004). Both DON and T-2 have been shown to impair host resistance and pulmonary immune responses to enteric reovirus infection in mice (Li et al., 2006; Li et al., 2007).

Ueno (Ueno, 1984) was the first to report on the inhalation toxicity of **T-2** in mice. Later, Creasia et al. (1987) demonstrated the acute effects immediately after inhaling aerosolic T-2 in mice: tremors, lethargy, stilted gait, and sometimes prostration. In rats and guinea pigs, respiratory exposure to T-2 revealed that lesions in histopathological examination were similar to those after systemic administration (Creasia et al., 1990). However, even after lethal doses, no histological evidence of acute pulmonary injury was found. The most consistent histological findings in guinea pigs after inhaled T-2 were lymphocytolysis and phagocytosis in the lymphoreticular system, and changes in the gastrointestinal tract (Marrs et al., 1986). Pang et al. (1987, 1988) reported that inhalation exposure of pigs to a sublethal dose of T-2 toxin caused morphological changes, clinical signs of toxicity and effects on hematology, serum biochemistry as well as on pulmonary and systemic immunity.

DON (vomitoxin) is a common trichothecene mycotoxin detected in grain dust. After nasal inhalation in mice, DON rapidly reaches the lungs and other organs, enhancing proinflammatory cytokine expression (Amuzie et al., 2008). Other effects of DON in multiple species are anorexia, diarrhea, growth retardation, neuroendocrine effects, and disruption of the immune system (Pestka, 2010a, 2010b). DON may be an indicator for other trichothecenes as DON, nivalenol and zearalenone have all been detected in the airborne dust from granaries. The occupational exposure to these toxins has been estimated to be below the tolerable daily intake (TDI) values, but activities such as cleaning have been shown to significantly increase the exposure of workers (Mayer et al., 2007; Niculita-Hirzel et al., 2016).

Satratoxins are acutely toxic secondary metabolites of *S. chartarum*, similarly as T-2 and HT-2 toxins. In mice models, the intranasal exposure to satratoxin G has been reported to evoke apoptosis of olfactory sensory neurons and acute inflammation in the nose and brain (Amuzie et al., 2010; Islam et al., 2006; Jia et al., 2011). Also, the nasal airways of rhesus monkeys were shown to be vulnerable to satratoxin G induced neuronal cell death and inflammation (Carey et al., 2012). Likewise, a single intranasal exposure to **roridin A** (a trichothecene mycotoxin with a similar chemical structure) has been found to induce rapid apoptosis and loss of olfactory sensory neurons in the nasal airways and the olfactory bulb in mice (Islam et al., 2007), and the effects may be even exacerbated by repeated exposure (Corps et al., 2010). These inflammatory effects have been suggested to account for nasal congestion and runny or itchy nose in people.

In addition to trichothecenes there is another mycotoxin family, **atranones**, which might contribute to the adverse health effects associated with *S. chartarum*. Pure atranones have been shown to contribute to inflammatory response by increasing the influx of immunocytes and cytokine responses in mice lungs after intratracheal instillation (Rand et al., 2006). It has been suggested that induction of inflammation by *S. chartarum* is related to atranones, while cytotoxicity to satratoxin production (Nielsen et al., 2002).

The mycotoxin research has been dominated by toxins of *Stachybotrys*, but it is good to keep in mind the wide **variety of other toxins** possibly relevant for human exposure. Less known "emerging toxins" such as sterigmatocystin and mycophenolic acid have been shown to occur frequently in agricultural products and thus could be relevant also for inhalation exposure (Gruber-Dorninger et al., 2016). There are indications that some MVOCs like 1-octen-3-ol might be a risk factor for indoor air related adverse health effects (Sahlberg et al., 2013). Toxins from *Penicillium* species common on wet building materials (brevianamide A, mycophenolic acid, and roquefortine C) have been shown to stimulate compound-specific inflammatory and cytotoxic responses in a mice model (Rand et al., 2005). Similarly, a study using doses comparable to the estimated doses of possible human exposure showed alterations in the expression of inflammation-associated genes in mice lungs after intratracheal instillation of toxins from fungi common on damp building environment (atranone C, brevianamide, cladosporin, mycophenolic acid, neoechinulin A & B, sterigmatocystin or TMC-120A) (Miller et al., 2010). A mixture of mycotoxins is also suggested to account for the ciliostatic activity of extracts of filamentous fungi isolated from mouldy buildings (Piecková and Kunová, 2002).

In addition to the reported inflammatory and cytotoxic responses, an increase of allergic immune response has also been linked with mycotoxin exposure. In a mice model of allergic asthma, the exposure to gliotoxin and patulin produced by *Aspergillus* and *Penicillium* species exacerbated the asthma-like phenotype by modulating the Th1/Th2 balance and by inducing oxidative stress (Schütze et al., 2010). However, in a cohort study measuring a broad set of microbial secondary metabolites no association between the presence of individual toxins and asthma development was observed, indicating that exposure to mycotoxins

is not responsible for the increased risk of asthma in homes with moisture damage and mold (Kirjavainen et al., 2015). Overall, the in vivo findings suggest that mycotoxins may play a role in acute inflammation of airways, neurological deficits and impaired host resistance to infections, which are all symptoms associated with damp building exposure. Thus, a prolonged exposure to mycotoxins may provoke some of the adverse health effects associated with damp indoor environments (Miller et al., 2010; Miller and McMullin, 2014; Rand et al., 2011).

10.4 Exposure Assessment

Adverse health effects of inhalation of microbial toxins are better known from occupational environments such as farms and feed manufacturing plants, where exposure to massive doses of organic dust and resulting pulmonary mycotoxicoses such as organic dust toxic syndrome (ODTS) have been well documented (Seifert et al., 2003; Viegas et al., 2013a). Establishing the link between inhaled microbial toxins in moisture-damaged indoor environments has proven to be more difficult, as the levels of microbial toxins are much lower and only few studies have characterized inhalation exposure accurately. Also the lack of specificity may cause problems, e.g. hundreds of MVOCs can be measured from the air, but none of these compounds are exclusively produced by microbes (Korpi et al., 2009). The exposure assessment is often based on presence of limited number of mycotoxins in indoor dust samples or biological fluids, whereas studies describing airborne concentrations of mycotoxins are virtually nonexistent (Tables 10.1 and 10.2).

Table 10.1 Clinical studies on adverse health effects related to mycotoxin exposure

Exposure assessment	Reference	*N* cases (+controls)	Country	Study population	Findings
Mycotoxins measured in indoor air or dust	Hayes et al., 1984	71 (+67)	The Netherlands	Oilpress workers	Aflatoxin exposure associated with total and respiratory cancer incidence
	Cai et al., 2011	462	Malaysia	Random sample of schoolchildren	Verrucarol in dust swabs associated negatively with daytime breathlessness
	Zock et al., 2014	645	Finland, Spain, The Netherlands	Schoolteachers from schools with and without moisture damages	Higher mycotoxin levels in settled dust associated with higher asthma symptom score and more nasal symptoms

(continued)

Table 10.1 (continued)

Exposure assessment	Reference	*N* cases (+controls)	Country	Study population	Findings
	Kirjavainen et al., 2015	228	Finland	Random sample of children	No association between levels of mycotoxins in floor dust with risk of asthma in children
	Norbäck et al., 2016	462	Malaysia	Random sample of schoolchildren	Verrucarol in dust swabs associated positively with tiredness
Mycotoxins or biomarkers measured from biological fluids or tissues of exposed persons	Dvorácková and Pichová, 1986	3	Czechoslovakia	Patients with pulmonary interstitial fibrosis	AFB1 detected in lungs of patients with interstitial fibrosis
	Trout et al., 2001	6 (+2)	USA	Hotel staff exposed to mold	Specific IgG antibodies to roridin in sera not related to exposure or the one diagnosed restrictive lung disease
	Croft et al., 2002	4	USA	Mycotoxicosis patients exposed to mold contaminated buildings	MT detected from urine of mycotoxicocis patients
	Rea et al., 2003	100	USA	Patients exposed to toxic molds in their homes	MT detected in urine of patients with health problems including respiratory and neurological deficits
	Dennis et al., 2009	79	USA	CRS patients with a history of mold exposure	MT detected from 7 out of 8 analysed urine samples
	Lieberman et al., 2011	18	USA	CRS patients undergoing endoscopic sinus surgery	OT detected in 22% of sinonasal tissue and secretions of CRS patients
	Thrasher et al., 2012	5	USA	Family of two adults and three children (and a pet dog) living in a water damaged home	MT, AT and OT in detected in urine, nasal secretions and tissue samples of patients with health problems including respiratory and neurological deficits

(continued)

Table 10.1 (continued)

Exposure assessment	Reference	*N* cases (+controls)	Country	Study population	Findings
	Brewer et al., 2013b	112 (+55)	USA	Patients diagnosed with CFS	MT, AT, OTA, levels in urine associated with CFS
	Brewer et al., 2013a	1	USA	Patient diagnosed with CFS	OTA detected in urine of CFS patient
	Malik et al., 2014	46 (+44)	India	Workers handling food grains	AFB1 in serum associated with occupational exposure and respiratory symptoms
	Lai et al., 2014	181 (+203)	China	Workers handling sugar and sugarcane	AFB1 albumin adducts in serum associated with hepatocellular carcinoma

AFB1 aflatoxin B1, *AT* aflatoxin, *CRS* chronic rhinosinusitis, *CFS* chronic fatigue syndrome, *MT* macrocyclic trichothecenes, *OTA* ochratoxin A, *OT* ochratoxin

Table 10.2 Biomonitoring of mycotoxin exposure in different exposure environments

Exposure assessment	Reference	*N* cases (+controls)	Country	Study population	Findings
Mycotoxins or biomarkers measured from serum of exposed persons	Autrup et al., 1991	45 (+45)	Denmark	Workers handling raw material for animal feed production	Levels of AFB1 adducts in serum were higher after 4 weeks of work compared to levels after vacation
	Iavicoli et al., 2002	6 (+23)	Italy	Workers processing coffee, black pepper, nutmeg and cocoa beans	OTA in serum associated with occupational exposure
	Van Emon et al., 2003	5 (+5)	USA	Individuals exposed to *S. chartarum* in water damaged environments	Stachylysin in serum associated with *S.chartarum* exposure
	Brasel et al., 2004	44 (+26)	USA	Individuals exposed to indoor molds	MT in serum associated with mold exposure (ELISA-assay)

(continued)

Table 10.2 (continued)

Exposure assessment	Reference	*N* cases (+controls)	Country	Study population	Findings
	Degen et al., 2007	61	Germany	Workers handling food grain	Levels of OTA in serum similar to average values in the German population
	Hooper et al., 2009	769 (+97)	USA	Individuals with or without known exposure to toxin producing molds	MT, AF and OT in urine, nasal secretions and tissue samples associated with reported mold exposure
	Oluwafemi et al., 2012	28 (+30)	Nigeria	Workers in animal feed production	AF in serum associated with occupational exposure and ventilation of the workplace
	Viegas et al., 2012	31 (+30)	Portugal	Workers in poultry production facilities	AFB1 in serum associated with occupational exposure
	Viegas et al., 2013b	28 (+30)	Portugal	Workers in swine production facilities	AFB1 in serum associated with occupational exposure
	Viegas et al., 2013c	45 (+30)	Portugal	Workers in swine (34) and poultry production (11)	AFB1 in serum associated with occupational exposure
	Viegas et al., 2015	41 (+30)	Portugal	Workers in waste industry	AFB1 in serum associated with occupational exposure
	Viegas et al., 2016	30 (+30)	Portugal	Workers in poultry slaughterhouse	AFB1 in serum associated with occupational exposure
	Föllmann et al., 2016	17 (+13)	Germany	Workers in grain mills	Levels of DON, OTA, zearalenone and citrinin in urine not associated with occupational exposure

AF aflatoxins, *AFB1* aflatoxin B1, *MT* macrocyclic trichothecenes, *OTA* ochratoxin A, *OT* ochratoxin

Determining mycotoxins directly from air is difficult, so usually the inhalation exposure is estimated by multiplying the mycotoxin concentrations in settled dust with either amount of dust measured from the air (Mayer et al., 2008) or derived from exposure limits for dust (Brasel et al., 2005). Even more often the exposure assessment is merely based on assumption that the mycotoxin exposure is taking place because mycotoxin producing fungi were detected from the indoor environment or exposure to molds was reported by the study subjects (Hodgson et al., 1998; Kilburn, 2009; Kilburn et al., 2009). Another shortcoming of mycotoxin exposure assessment is the lack of information about the "normal" level of mycotoxin exposure in indoor environments, although mycotoxins are known to be ubiquitous in built environment and found also from outdoors (Peitzsch et al., 2012; Täubel et al., 2011).

10.5 Biomonitoring of Mycotoxins

Mycotoxins, specifically trichothecenes, aflatoxins, and ochratoxins, can be detected in human tissue and body fluids in patients who have been exposed to toxin producing molds in their environment (Table 10.2). In fact, in a study of Hooper et al. (2009) trichothecenes were found in 95% of urines, 44% of nasal secretions and 59% of tissue, whereas aflatoxins and ochratoxins were present in lesser cases. Only one study reports an association between levels of OTA in serum and occupational exposure, but AFB1 levels in serum have been consistently associated with working in waste industry, poultry farms and slaughterhouses (Oluwafemi et al., 2012; Viegas et al., 2012, 2013b, 2013c, 2015, 2016). Similarly, levels of macrocyclic trichothecenes as well as hemolysin stachylysin in serum have been associated with exposure to indoor molds (Brasel et al., 2004; Van Emon et al., 2003). Few studies report no association between occupational exposure and levels of mycotoxins in biological fluids (Degen et al., 2007; Föllman et al., 2016), suggesting that exposure levels can be controlled even in workplaces with high risk for mycotoxin contamination.

Some mycotoxins can also form metabolites or protein adducts, which could serve as biomarkers of exposure; e.g. intratracheal exposure to satratoxin G produced adducts with serum albumin in both humans and rats (Yike et al., 2006). Serum AFB1 albumin adducts have been used to assess chronic exposure via both diet (Leong et al., 2012) and inhalation (Autrup et al., 1991; Lai et al., 2014), but detectable levels of adducts were seen only after inhalation exposure to a certain level of AFB1 in an epidemiological study and in an animal model (Mo et al., 2014). Generally, several biomarkers of oral exposure exists for the agriculturally important toxins (Lee and Ryu, 2015), but for the toxins from building associated fungi there is hardly any biomarkers available. The development of biomarkers showing inhalation exposure to broader set of mycotoxins would be an important step for risk assessment.

10.6 Risk Assessment

A valid risk assessment for inhalation exposure to microbial secondary metabolites is a demanding task for several reasons. Firstly, accurate exposure assessment is needed since mycotoxins can show a wide variety of effects (e.g. immunotoxic, carcinogenic, mutagenic, toxic or teratogenic) depending on the type of toxin. The type of exposure (inhalation, oral, dermal) may also affect the adverse effects seen; the inhalation route seems to be more harmful than ingestion of certain mycotoxins. The dose and duration of exposure are important determinants as well as the toxin fraction absorbed after respiratory intake. Furthermore, the sensitivity to mycotoxins varies between exposed individuals or population groups (Fromme et al., 2016; World Health Organization, 2009). Worryingly, children appear to be at greater risk for inhaled toxicants, as the respiratory deposition of fungal fragments into to lower airways of infants was found to be four to five times higher than in adults (Cho et al., 2005).

In indoor environment toxins never exist individually, but rather are mixed with a variety of fungi and bacteria, their spores, fragments and cell wall components together with allergenic proteins, volatile organic compounds and particulates (Nevalainen et al., 2015). In experimental setups, mycotoxins have been shown to interact synergistically with each other (Vejdovszky et al., 2017), with microbes (Huttunen et al., 2004; Penttinen et al., 2005), with spores (Šegvić Klarić et al., 2015), with microbial structural components (Islam and Pestka, 2006; Islam et al., 2007; Kankkunen et al., 2009, 2014; Korkalainen et al., 2017; Zhou et al., 1999) as well as with particulate matter (PM_{10}) (Capasso et al., 2015). Similarly, there is evidence of strong synergistic or additive effects after simultaneous exposure to multiple foodborne toxins (Stoev, 2015). Synergistic effects of microbial secondary metabolites should be given due consideration, as there is a possibility of underestimating the effects of microbial exposures if the risk assessment is based on information on individual exposure agents.

Several regulatory agencies and organizations, such as World Health Organisation (WHO), Joint FAO/WHO Expert Committee on Food Additives (JECFA), EU Scientific Committee for Food (SCF) and European Food Safety Authority (EFSA) have set maximum permissible levels and produced TDIs for agriculturally important mycotoxins worldwide. International Agency for Research of Cancer (IARC) has also evaluated the carcinogenic risks of some naturally occurring toxins to humans. However, these limit values are merely directed to regulate the exposure via food. Since only limited toxicological data from inhalation exposure are available, the extended threshold of toxicological concern (TTC) concept (concentration of no toxicologic concern, CoNTC) has been introduced for safety assessment purposes (Drew and Frangos, 2007; Munro et al., 2008). The resulting airborne concentration, 30 ng/m^3, is expected not to pose hazard to humans through lifetime. Although exposures in the most extreme working conditions may exceed this concentration, measured airborne mycotoxin levels in the built indoor environment have been repeatedly found to be too low to

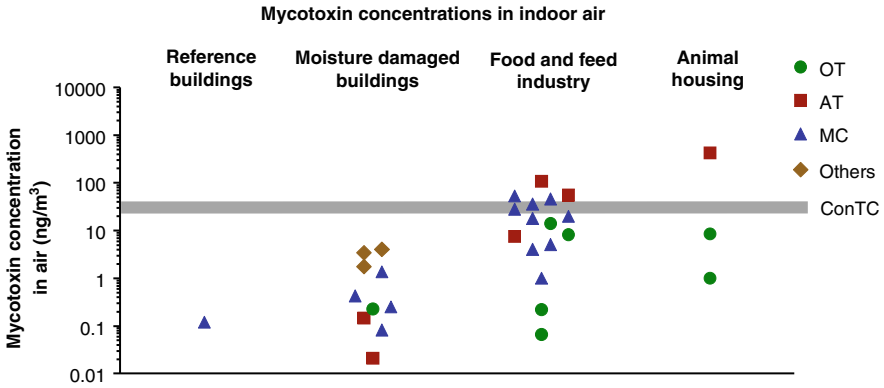

Fig. 10.2 Airborne concentrations of common mycotoxins ochratoxin (OT), Aflatoxins (AT), macrocyclic trichothecenes (MC) and other mycotoxins (Others) in different indoor environments, including reference buildings, moisture damaged buildings (Brasel et al., 2005; Gottschalk et al., 2008; Polizzi et al., 2009), food and feed industry (Burg et al., 1981, 1982; Iavicoli et al., 2002; Niculita-Hirzel et al., 2016; Sorenson et al., 1984) and animal housing (Selim et al., 1998; Skaug et al., 2001; Wang et al., 2008). The concentrations are either measured directly from air or measured from a dust sample and airborne concentration is calculated assuming a dust concentration of 15 mg/m^3. The grey line represents the concentration of no toxicologic concern (ConTC) for mycotoxins showing the level of exposure not considered to be hazardous even with lifetime exposure (Hardin et al., 2009)

pose a credible health risk, as the doses of both nonvolatile and volatile microbial metabolites are consistently below the CoNTC (Hardin et al., 2003; Hardin et al., 2009; Kelman et al., 2004; Robbins et al., 2000, Korpi et al., 2009) (Fig. 10.2). However, the presence of mycotoxins in materials or products handled in a workplace should be considered as a potential hazard to health, even though the causal connection between mycotoxin exposure and adverse health effects is not established and risk assessment requires more detailed information about the quality and quantity of the inhaled substance (Degen, 2011).

10.7 Conclusions

Microbial secondary metabolites can be found from indoor environments and particularly in workplaces where organic material is handled or moisture damage supports excessive microbial growth. Exposure to microbial toxins is possible and even likely, as the spores and fragments of microbes can act as carriers. Among microbial secondary metabolites there are numerous compounds which have been shown to be toxic to mammals. However, the concentrations of microbial toxins indoors are typically low, and same microbial secondary metabolites can be found also from outdoor air as well as microbiologically “normal” indoor environments.

In order to pose a health risk the amount of toxin in the air (or retained in the body) should exceed the threshold of toxicological concern, and at least for single toxins, this is typically not the case.

Future efforts should concentrate on the studies of multiple exposures, comorbidities of microbial toxicoses and effects of low-level chronic exposures. Epidemiological studies are needed to establish causality, but they need better measures of exposure to a wider selection of microbial secondary metabolites. There is a lack of toxicokinetic data on humans, clinical studies measuring both exposure and health outcome as well as biomonitoring studies assessing internal exposure after respiratory intake in residential settings. In addition, more experimental studies with advanced models of respiratory system are needed to elucidate the complex relationships between toxin exposure and health outcome.

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